Phosphorus sensitivity in species of Proteaceae (*Protea obtusifolia*; *Leucadendron coniferum; and Leucadendron salignum*) from different soil habitats:

Possible candidates for growth on former agricultural soils high in P

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by
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Ecophysiology

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Anti-plagiarism Declaration

I hereby declare that this project is my own work and that any other ideas or input that were not my own were referenced and/or acknowledged. This thesis is towards completing my honours degree at the University of Cape Town and will not be used for any other purposes.

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Abstract

The phosphorus (P) uptake capacities and tolerance to high P of three Proteaceae species from acid (*Leucadenron coniferum*, *Leucadendron salignum*) and alkaline (*Protea obtusifolia*, *Leucadedndron meridianum*) soils in the Cape Floristic Region (CFR) were compared. These species were also investigated as potential candidates for growth on nutrient enriched, post-agricultural soils. In parallel, two crop plant species (*Lupinus albus,* with cluster roots; and *L. angustifolius*, without cluster roots) were also compared. The cluster-root forming species (*L. albus*) was more sensitive to higher P levels and showed toxicity in terms of photosynthesis, efficiency of PSII, transpiration and stomatal conductance. Growth, gaseous exchange and efficiency of photosystem II (as a measure of stress) were determined at 1, 10 and 100 μg P g^{-1} sand for both lupins and Proteaceae. Kinetics of P uptake (in hydroponics) was determined at a range (4 to 2000 μM P) of P concentrations. Phosphorus sensitivity was relatively high in *Ld. coniferum* as evident from reduced growth, net photosynthetic rate, stomatal conductance and transpiration rates. *Protea obtusifolia* showed the least sensitivity to increased phosphorus concentrations under acidic and native alkaline conditions. The inability to regulate P uptake rates at elevated phosphorus concentrations and excessive P accumulation were major determinants of P sensitivity. The relatively higher P sensitivity of *Lupinus albus* compared to *L. angustifolius* is likely related to the efficient P uptake by cluster roots in the former.

Introduction

The South African fynbos biome, which is part of the Cape Floristic Region (CFR), is known to have nutrient poor soils, in particular very low levels of inorganic phosphorus (in the range of 0.4 to 8.3 μg g^{-1} soil dry mass) (Bond and Goldblatt, 1984; Witkowski and Mitchell, 1987) and inorganic nitrogen (3-6 mg kg^{-1} soil depending on soil age; Hawkins *et al.*, 2005). Up to 60% of total P in the CFR soils can be organic (Mitchell et al., 1984). Inorganic P varies from Ca-bound to Al and Fe-bound forms in the different soil types (Witkowski and Mitchell, 1987). In neutral to alkaline soils, P ions will precipitate as Ca phosphate and in acidic conditions P ions will precipitate as Fe and Al phosphates (Hinsinger 2001). Phosphorus-binding forms may be one of the major determinants in the distribution of vegetation types along

with the differences with respect to texture, pH and absolute nutrient content (Mustart and Cowling 1993). Cowling and Mustart (1993) used the differential seedling growth and mortality as evidence for adaptations in limestone and acidic soil species to their native soil types.

The ability of fynbos species to flourish in low P soils depends on plant physiological as well as morphological adaptations to these low concentrations of phosphorus. For example, many Proteaceae species form cluster-roots which exude acids to increase the availability of P in the rhizosphere by altering the rhizosphere pH and chelating cations by ligand exchange (review by Shane and Lambers 2005). Another adaptation is at the level of transcription of high affinity phosphate transporters, inducible at low soil [P] (Raghothama and Karthikeyan 2005). The result of agricultural activities in the south-western Cape is that large areas of fertilised soils are unsuitable for indigenous vegetation. There is a reduced growth in the grain and fruit growing industries in this region and an increasing growth in e.g the indigenous Proteaceae cut-flower industry. As a result, farmers are looking for suitable species to grow on previously agricultural land. The major challenge is to match the nutrient requirements of plants to the nutrient status of these soils, since fertilisation results in raised levels of phosphorus (P) and P is leached out of these soils very slowly since phosphate move through the soil via diffusion and not via mass flow (Marschner, 1995). In addition, P binds strongly to cations such as Ca and Fe, which are abundant in the Cape Floristic Region (CFR) (Mitchell et al., 1984)

The availability of inorganic P to plants is dependent on complex interactions between internal and external regulation of [P], which is greatly influenced by the soil pH since P is more soluble in acidic soils. Furthermore, cluster-root exudates, usually acidic, play a significant role in the availability of P, by further modifying the pH. For example, root induced acidification of the rhizosphere, i.e., the release of H^+, increases the bioavailability of inorganic P in alkaline soils in the presence of Ca-phosphates. In the absence of Ca-phosphates, i.e. acidic soils, a further decrease in the pH by root exudates leads to a decrease in the availability of inorganic phosphorus for plant uptake (Hinsinger, 2001). Enhanced P uptake and modifications of P availability by Proteaceae enable these plants to survive in low-P soils but the same mechanisms can easily lead to P toxicity in plants as was the case for Australian Proteaceae (Shane

et al 2004). The high sensitivity of certain species to even the slightest increase in P levels was found to be mainly due to the inability to regulate P uptake and/or to store exceedingly high concentrations of phosphorus in the roots during the non-growing season (Shane and Lambers 2006).

Ecological studies showed that *Leucadendron meridianum* had poor or no seedling survival when transplanted into non native soils (acidic soils) whereas *L. coniferum* showed a greater survival on the limestone soils than on their native acidic soils (with the exclusion of competition). Nonetheless, *L. coniferum* had a greater biomass on their native soils (Mustart and Cowling, 1993). This study expands on these previous findings to offer an ecophysiological insight to species distribution. The P sensitivity of three Proteaceae that vary in soil pH preference (*Protea obtusifolia* (limestone species), *Leucadendron coniferum* (acid species) and *Leucadendron salignum* (generalist species)) was assessed. The hypothesis was that the limestone species, *Protea obtusifolia*, would be the most suited to high-P soils since this species occurs on soils with naturally higher P levels and therefore may have lower affinity phosphate transporters or possibly higher plasticity for P storage in roots and allocation to growth (as was the case for *Grevillea crithmifolia* in a study by Shane and Lambers 2006). It was expected that the acid-loving or calcifuge species, *Ld. coniferum*, will be the least tolerant of high [P] but the most sensitive and efficient regarding acquisition of P at low P supplies, while *Ld. salignum*, the generalist, will be intermediate between the other two species. In parallel with these studies, the P sensitivity of two crop plants *Lupinus albus* (a cluster root forming lupin) and *Lupinus angustifolius* (a non-cluster root forming lupin) was determined. It was predicted that the cluster-root forming species (*L. albus*) will be more sensitive to higher P levels and show toxicity in terms of photosynthesis, efficiency of PSII, transpiration and stomatal conductance.

Methods and Materials

Glasshouse experiments were conducted to determine P sensitivity in Proteaceae plants (*Protea obtusifolia*, *Leucadendron salignum*, *Leucadendron coniferum* (Proteaceae)) and Fabaceae plants (*Lupinus albus*, *Lupinus angustifolius*) in sand with either acid or alkaline pH at three different phosphorus concentrations. Apart from P,

other nutrients were also added to all treatments to approximate concentrations in CFR soils. P sensitivity was assessed in terms of growth, efficiency of photosystem II (as an indicator of stress) and gaseous exchange. Hydroponic experiments were conducted to determine P-uptake kinetics of the three species at pH 4.5 and 7.5 and various P-levels.

Plant cultivation

Seedlings of *Leucadendron coniferum* and *salignum* were kindly provided by Monique Twine and Anthony Hitchcock of the South African Biodiversity Institute (SANBI) nursery, Kirstenbosch, Cape Town, SA. The one-year old seedlings had been grown from seed collected in 1975 and were grown in the standard mix used by the nursery (Table Mountain Sandstone sand and bark). One year old *Protea obtusifolia* seedlings were obtained from Good Hope Nursery, Simon's Town and were grown in a standard mix. Plants were transplanted as described below and cultivated in a greenhouse where the average day temperature was 25°C and the average night temperature was 17°C.

Proteaceae seedlings (n=5) and Lupin seedlings (n=5) were transplanted into 18 cm pots containing 2 kg sand (1:1 of 2mm and 0.5 mm sand, Consul). Poorly soluble phosphate ($CaHPO_4$) was pre-mixed into the sand using a cement mixer to levels of 1, 10 and 100 µg P g^{-1}sand dry mass. Prior to transplanting, plant roots were carefully washed free of soil. All three Proteaceae species were grown at three phosphorus concentrations and two pH levels (pH 4.5 and 7.5) thereby exposing each species to its natural soil conditions and to contrasting conditions. Lupin species were grown at three phosphorus concentrations and pH 6.5. To obtain the desired pH, a complete nutrient solution (modified, P-free ¼ strength Hoaglands) consisting of: $Ca(NO_3)_2.4H_2O$ (0.602 µM); CaSO4.2H2O (0.200 µM); ; K_2SO_4 (0.300 µM); $MgSO_4.7H_2O$ (0.108 µM) $MnSO_4.H_2O$ (0.239994 µM); $ZnSO_4.7H_2O$ (0.102 µM); $CuSO_4.5H_2O$ (0.018 µM); H_3BO_3 (2.400 µM); $Na_2MoO_4.2H_2O$ (0.030 µM); FeEDTA (10.116 µM), pH buffered to 4.5, 6.5 or 7.5 with 2mM MES-KOH, was added at each watering event (every other day, 100ml per pot). The respective P concentrations were set with reference to the natural levels of these different pH's, as found in a study by

Witkowski and Mitchell (1987). In their study, De Hoop soils (representing limestone soils) had Bray no. 2 levels of about 10 μg P g^{-1} dm, while Bainskloof soils (representing acidic soils) had Bray no. 2 levels of about 1 μg P g^{-1} dm. The highest P concentration, set at 100 μg P g^{-1} dm was found to be toxic to most Proteaceae species on former apple orchard soils of Molteno Bros. (Hawkins, pers com).

Plant growth determination

Growth of plants in sand was determined non-destructively by determining stem length at 0, 1, 2, 3 and 4 months. Growth was calculated as the maximum minus minimum growth over the 4 month growth period. It was intended to determine the micro-location of elements (Fe, Mg, Mn, Ca and P) using proton induced x-ray emission (PIXE) analysis of leaf samples prior to harvest. Unfortunately, plants were lost prior to this.

P uptake kinetics

Five individuals per species (both lupins and Proteaceae) were grown in hydroponics (modified, ¼ strength Hoaglands, 10 μM soluble P in the form of KH_2PO_4, pH 6.5) for 3 weeks (lupins) or 2 months (Proteaceae). Since acquiring the desired number of *P.obtusifolia* plants for both glasshouse and hydroponic experiments was not possible, *Leucadendron meridianum* was used as a replacement species for *Protea obtusifolia.* These two species are similar in their pH and phosphorus preferences. Phosphorus uptake at varying P concentrations was measured as P depletion in order to determine the Michaelis-Menton kinetic parameters I_{max} and K_m. Root pieces from five plants were divided into six 50 ml beakers containing 30 ml of modified, ¼ strength Hoaglands with 1, 5, 10, 50, 100, 500 or 1000 μM KH_2PO_4 at pH 6.5. The pH was maintained with 2 mM MES-KOH. Each vial containing the root material was kept aerated throughout the course of uptake kinetics by gently shaking it. After three hours, the solutions were filtered and used to determine the phosphorus concentration by using the protocol in Diatloff and Rengel (2001). Spectrophotometer readings (at 650 nm) were done in a microtiter plate format..

Gaseous exchange and leaf fluorescence

Leaf fluorescence parameter and gaseous exchange were measured simultaneously using LI-6400 portable photosynthesis system (LI-COR Biosciences inc. Nebraska, USA). Leaf fluorescence of the photosystem II (ΦPSII) is the fraction of absorbed photosystem II (PSII) photons that are used in photochemistry. It is calculated from Fs (steady-state fluorescence) and Fm (the maximum fluorescence from a light-adapted sample upon application of a saturation flash (Maxwell and Johnson, 2000). The efficiency of PSII is commonly used as an indication of physiological stress (Maxwell and Johnson, 2000) and was used to determine the effect of excess P on PSII. The following acronyms are used in the text: Net photosynthetic rate (P_{sn}), Efficiency of photosystem II (ΦPSII), Stomatal conductance(G_s) and Transpiration (T_{pn}). Not standard

Statistical analyses

Due to heteroschedastic data, phosphorus uptake rates and phosphorus concentrations were log transformed prior to statistical analyses. The data were analysed using the Newman-Keuls multiple range tests after two-way ANOVAs with pH and P supply as factors for each species or one-way ANOVAs with P supply as a factor.

Results

The effect of phosphorus concentration and pH on growth and biomass allocation

Neither P nor pH had a significant effect on growth of the three Proteaceae species (Table 1). However, there was an obvious trend towards decreasing biomass allocation with increasing P supply in all Proteacea under native pH conditions (Table 1). The external phosphorus concentration affected growth in *Leucadendron coniferum* significantly ($p=0.009$, two-way ANOVA) and there was a substantial decrease in growth between 10 and 100 μg P g^{-1} sand in both alkaline and acidic conditions.

Table 1. Effect of pH and P concentration on growth (stemlenght (cm)), measured in three Proteaceae species (over a four month growing period) at three phosphorus concentrations per alkaline and per acidic sand medium. Values in parantheses indicate the standard errors and letters indicate significant differences at p<0.05 (Two-way ANOVA with post-hoc Newman-Keuls multiple range test, n = 4 or 5).

Species	Phosphorus concentration (µg P g^{-1} sand)	pH	
		Acidic (4.5)	Alkaline (7.5)
Protea obtusifolia (limestone sp.)	1	8.1 (0.8) a	8.1 (1.1) a
	10	7.8 (0.8) a	9.2 (1.9) a
	100	7.2 (1.0) a	4.1 (3.0) a
Leucadendron salignum (generalist sp.)	1	12.0 (3.0) a	8.7 (1.8) a
	10	12.2 (2.3) a	10.8 (1.4) a
	100	7.5 (2.0) a	12.6 (2.7) a
Leucadendron coniferum (acidic sp.)	1	17.9 (2.2) a	15.7 (1.6) a
	10	18.3 (1.2) a	15.0 (1.8) a
	100	9.6 (3.1) a	9.3 (3.3) a

In both lupins, phosphorus toxicity symptoms appeared at 100 µ P g^{-1} sand after four weeks, but were more severe in the cluster-root forming species (*L. albus*) than in *L. angustifolius*. The external phosphorus concentration had no significant effect on the biomass allocation of *L. angustifolius*. As expected, biomass allocation to cluster roots in *L. albus* was significantly higher (p=0.008, one-way ANOVA) at the lower P concentration (1 µg P g^{-1} sand) and comprised almost half of the total root biomass at this concentration.

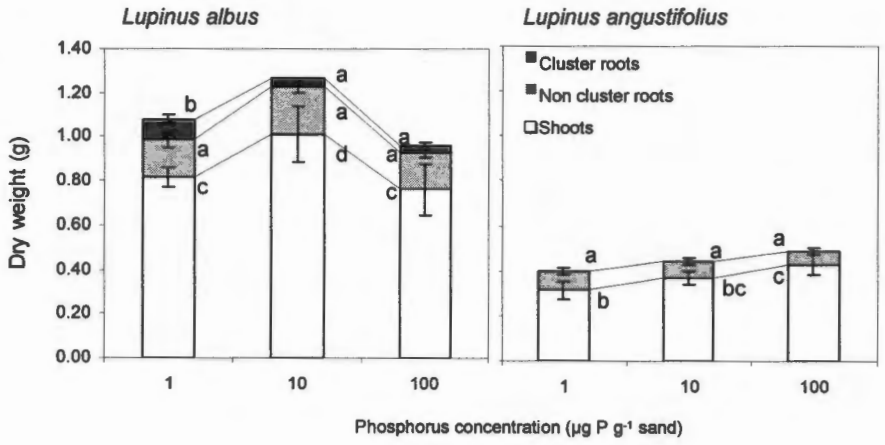

Fig. 1. Effect of P concentration on biomass allocation in *L.albus* (cluster root forming species) and *L.angustifolius* (non cluster root forming species). Letters indicate significant differences at p<0.05 (two way ANOVA and one-way ANOVA with post hoc Newman-Keuls multiple range testvalues are means of 5 replicates +/- std error)

The effect of P concentration and pH on gaseous exchange

The general trend in gaseous exchange of *Protea obtusifolia, Leucadendron salignum and Leucadendron coniferum* was similar and all three species reached a maximum at 10 μg P g^{-1} sand, regardless of the pH of the medium (Fig 2). However, there were differences regarding the independent effects of pH and P concentration and the interaction of these factors on the various components of gaseous exchange in the three species. Gaseous exchange and efficiency of PSII in *P.obtusifolia* (limestone species) and *Ld. coniferum* (acidic species) reached a maximum under native pH conditions, while the generalist species (*Ld. salignum*) functioned optimally under alkaline conditions.

Photosynthetic rate, ΦPSII, T_{pn} and G_s in *P.obtusifolia* was significantly affected by pH (p=0.001, p = 0.03, p = 0.05 and p = 0.04 respectively, two-way ANOVA). While phosphorus concentration did not significantly affect P_{sn}, there was a significant effect on both T_{pn} and G_s (p = 0.05 and p = 0.03 respectively, two-way ANOVA) resulting in a considerable decrease in rates at the lowest and highest P concentration. The interaction of [P] and pH had a significant (p = 0.025, two-way ANOVA) and contrasting effect on ΦPSII at the highest P concentration; under alkaline conditions ΦPSII decreased opposed to increasing efficiency under acidic conditions

Leucadendron salignum showed considerable variation in minimum and maximum gaseous exchange rates at the different P concentrations and between different pH conditions. P_{sn} was significantly affected by pH (p = 0.013, two-way ANOVA) and P concentration (p=0.049, two-way ANOVA) and there was interaction between the two factors (p=0.023, two-way ANOVA). Like *P. obtusifolia*, P_{sn} in *Ld. salignum* reached the highest optimum in alkaline conditions but stomatal conductance and transpiration rates were highest under acidic conditions (at 10 μg P g^{-1} sand). In *Ld. salignum*, not only phosphorus concentration but the interaction of P and pH significantly affected rates of stomatal conductance, transpiration and efficiency of photosystem II ($p<0.05$, two-way ANOVA). In all of these functions, particularly the efficiency of PSII, there was a significant decline at 100μg P g^{-1} sand in acidic conditions but not in alkaline conditions where the effect was largely negligible between 10 and 100μg P g^{-1} sand,

but more obvious in the increase in rates and efficiency of PSII between 1 and 10 μg P g^{-1} sand (fig 2).

In *Leucadendron coniferum*, gaseous exchange rates (except photosynthetic rate) were mainly affected by the P concentration of the medium as apposed to pH. As expected, there were maximum P_{sn} rates and ΦPSII under acidic conditions rather than in alkaline conditions. In alkaline conditions the minimum was reached at the highest P concentration (100 μg P g^{-1} sand), whereas in acidic conditions it was reached at the lowest [P] (1 μg P g^{-1} sand). The patterns of stomatal conductance and transpiration corresponded to the trend in photosynthetic rate, and was significantly affected by P concentration (G_s, $p = 0.05$, T_{pn}, $p = 0.04$, two-way ANOVA). The efficiency of PSII was significantly affected by the P concentration and not pH; for both pH levels the minimum was reached at the highest [P] (100 μg P g^{-1} sand $^{-1}$).

The effect of P concentration on gaseous exchange in the crop species differed remarkably between a cluster root and non-cluster root forming species (Fig. 3) and although net P_{sn}, ΦPSII, T_{sn} and G_s of *Lupinus albus* (cluster root forming) were not significantly affected by P concentration, this species showed substantially higher rates than *Lupinus angustifolius* (non-cluster root forming).

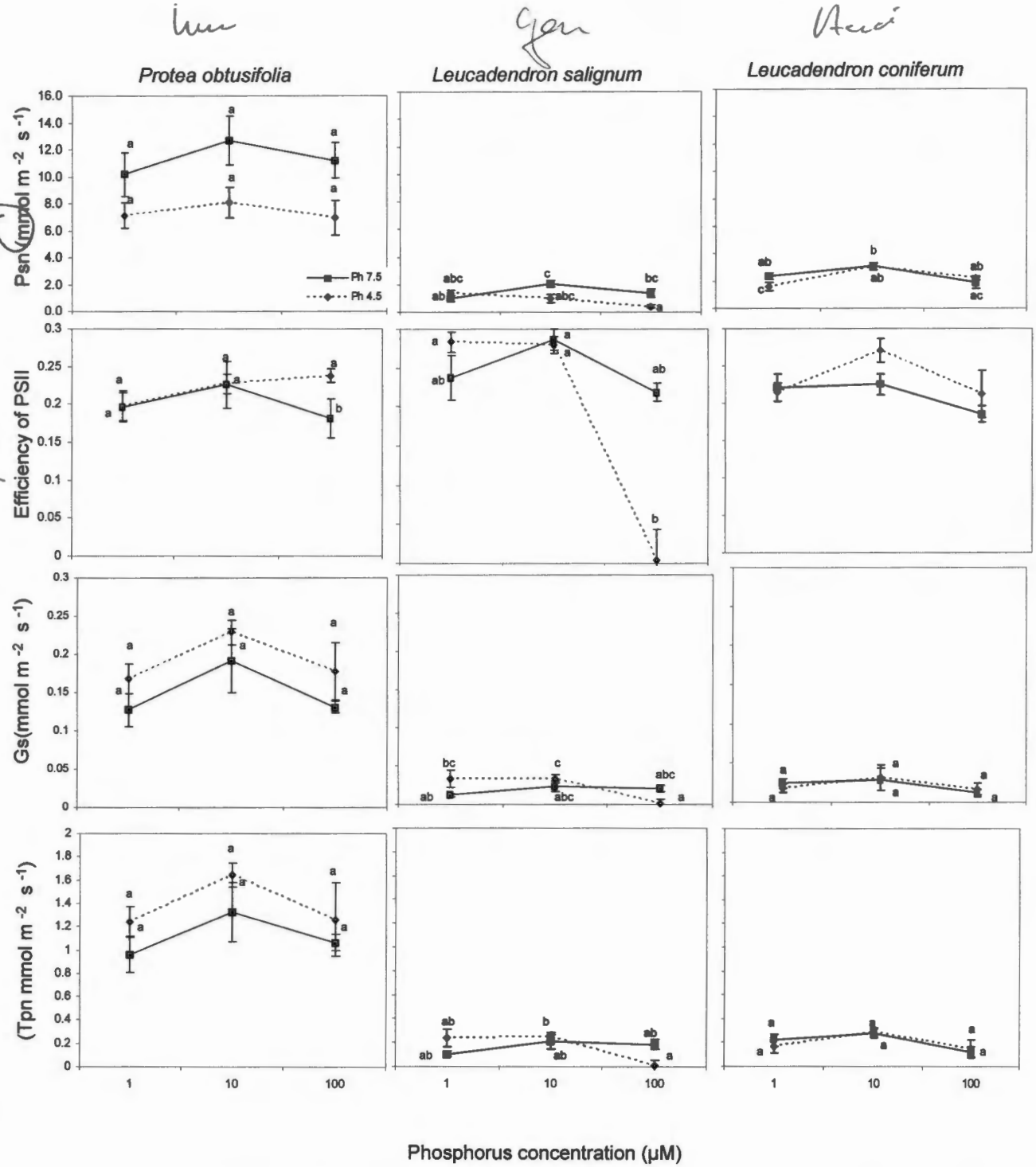

Fig. 2 Effect of phosphorus concentration and pH (♦- - - pH 4.5 ■—pH 7.5) on gaseous exchange of three Proteaceae species. Letters above error bars indicate significant differences at $p<0.05$ (two-way ANOVA with post hoc Newman-Keuls multiple range test. Values are means of 8,9 or 10 replicates +/- std errors).

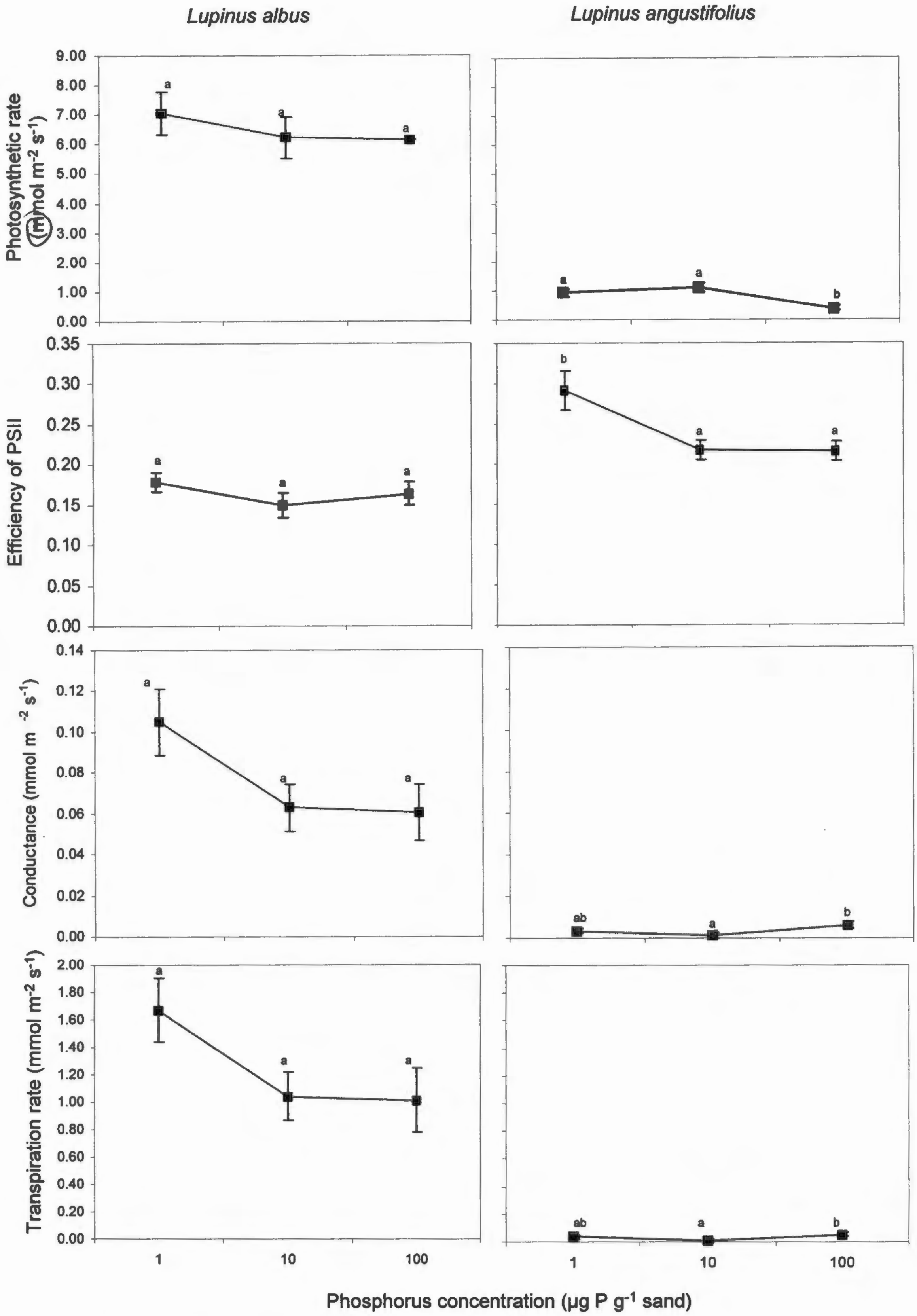

Fig. 3. Effect of phosphorus concentration on gaseous exchange of a cluster root forming (*L.albus*) and a non-cluster root forming (*L.angustifolius*) crop species. Letters above error bars indicate significant differences at $p<0.05$ (one-way ANOVA with post hoc Newman-Keuls multiple range test. Values are means of 9 or 10 replicates +/- std errors).

How was this possible? L. ang. did not get P!!

3. The effect of external phosphorus concentration and cluster roots on the efficiency of phosphorus uptake

Cluster root formation commenced in both *Leucadendron meridianum* and *Leucadendron coniferum* after 2 months of growth in hydroponic solution (low in external phosphorus supply), and were well developed by 3 ½ months when they were used for assays (Appendix A, Fig.1). However, *Leucadendron salignum* had not formed any cluster roots at that stage. Phosphorus uptake in all of the species showed a constant and significant increase (*Ld. meridianum* and *Ld coniferum* $p<0.001$; *Ld. salignum* $p<0.0001$, two-way ANOVA) at external P supplies ranging from 10 to 2000 μM and did not reach a maximum rate at the highest external P concentration.

In *Ld. meridianum*, non-cluster root uptake rates were significantly higher ($p <0.001$ two-way ANOVA) than cluster root uptake rates while in *Ld. coniferum* cluster root uptake of P was significantly greater than non-cluster root uptake ($p<0.001$, two-way ANOVA). Uptake by *Ld. salignum*, which had not formed cluster roots, was comparable to non-cluster root uptake of P by the other two species (Fig. 4).

In the crop species, *Lupinus albus* showed no significant differences in P uptake rates between cluster and non-cluster roots, but the uptake rates in both root types were significantly affected by the external P supply ($p<0.001$, two-way ANOVA). Cluster roots released phosphorus at the intermediate P concentration (Appendix A, table 1). In the non-cluster root forming species (*L. angustifolius*), uptake rates were significantly different at the three external phosphorus concentrations ($p <0.001$, two-way ANOVA) and this species appeared to have a higher uptake rate than the non-cluster root forming species at the highest external P concentration (2700 μM P). At the lowest P concentration (14 μM P) the uptake rates were very similar for both species (Fig 5).

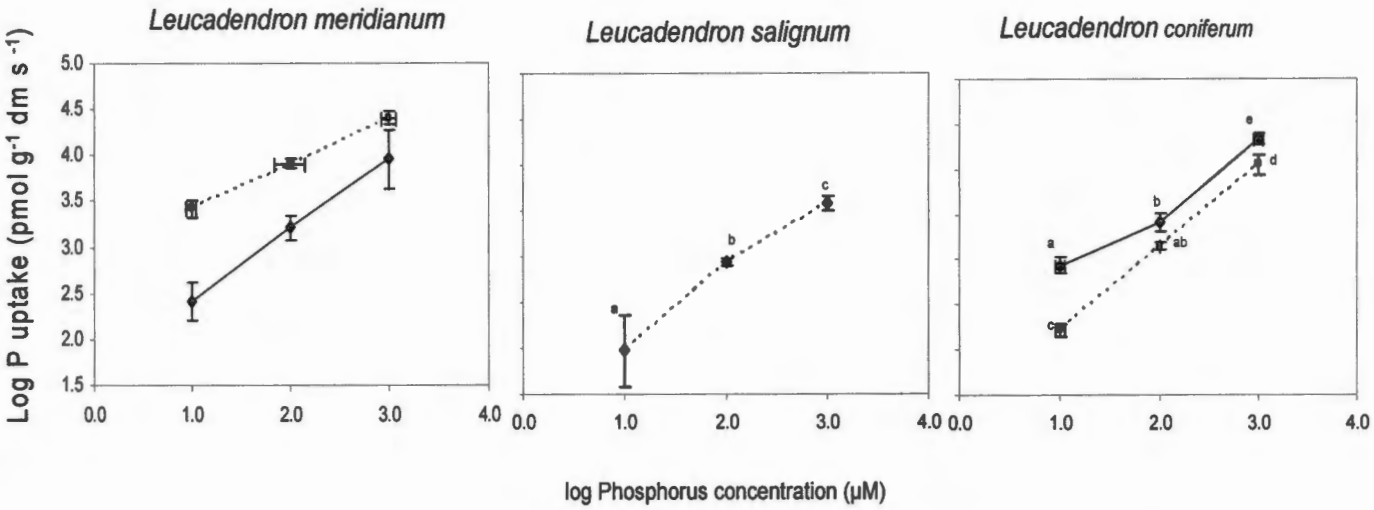

Fig. 4. The phosphorus uptake rates of cluster (—♦—) and non-cluster roots (---■--) of three Proteaceae species at different external supplies of phosphorus concentrations. Letters above error bars indicate significant differences at $p<0.05$ (Two-way ANOVA for *Ld. meridianum and Ld coniferum*; One-way ANOVA for *Ld. salignum* with post hoc Newman-Keuls mulitple range test. Values are means of 4 or 8 replicates +/- std errors).

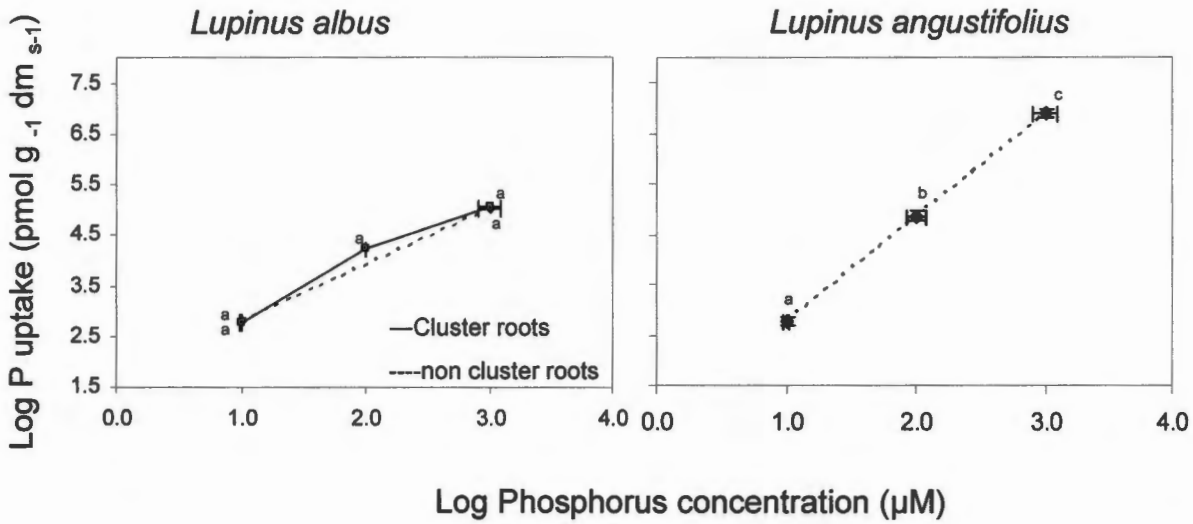

Fig. 5. Phosphorus uptake rates in a cluster root forming (*L.albus*) and non-cluster root forming (*L. angustifolius*) crop species. Letters above error bars indicate significant differences at $p<0.05$ (Two-way ANOVA for *L.albus* and one-way ANOVA for *L. angustifolius*. Values are means of 8 or 12 replicates +/- standard errors).

Discussion

The effect of P concentration and pH on growth

Irrespective of P or pH treatment *Protea obtusifolia* did not grow well and it is thought that this was the result of transplant sensitivity rather than treatments per se. The notable but non significant decrease in growth at 100 µg P g^{-1} sand in *Leucadendron salignum* under acidic conditions may be ascribed to the corresponding significantly lower P_{sn} at 100 µg P g^{-1} sand. The lower P_{sn} was linked to reduced G_s and T_{pn}. Somatal conductance may have been reduced by P-binding of Ca. A reduction in freely exchangeable cytosolic Ca^{2+} affects the action of ABA (abscisic acid) on stomatal closure (Marschner 1995). Apart from P-binding of Ca, it was found that Ca concentrations in a Proteaceae cultivar 'Safari Sunset' was higher in epidermal tissues of control plants than plants supplied with up to 5mM P (Hawkins 2006, unpublished) and this is likely relevant to most Proteaceae species. In *Leucadendron coniferum* the effect of external P on internal processes is only significant at 100µg P g^{-1} sand, but under both acidic and alkaline conditions and this could largely be attributed to this species' natural occurrence in acid soils particularly low in P. With increased P availability through enhanced P uptake through cluster roots, this species could suffer P toxicity more severely than the other species studied.

Increased biomass allocation in the cluster root forming lupin species (*L.albus*) with low P supply confirms previous findings of an inverse correlation of cluster root allocation with leaf [P] (Keerthisinghe *et al.* 1998). Furthermore, in *L. albus* and *L. angustifolius* shoot growth was more inhibited by P deficiency than root growth which confirms previous findings (Marschner 1995). The greater effect of P concentration on biomass allocation in the cluster root forming species compared to the non-cluster root forming species is likely to be related to uptake efficiency and P storage as explained in more detail in sections to follow.

The effect of P concentration and pH on gaseous exchange

This study revealed that P concentration had a major effect on Proteaceae species while pH did not. In previous studies, P deficiency had lead to impaired carboxylation efficiency consequently affecting CO_2 assimilation (Pieters *et al.* 2001). On the other hand, excess phosphorus supply to plant tissues has been shown to increase P accumulation in vacuoles of palisade cells which in return resulted in decreased photosynthesis (Shane *et al.* 2004a). The effects of both P deficiency and P toxicity on Proteaceae and crop species are discussed below.

Protea obtusifolia is adapted to soils naturally richer in phosphorus but elevated P concentrations under acidic conditions substantiates Cowling and Mustarts's (1993) findings of poor seedling performance under non-native conditions. Ecophysiological explanations for the inconsistent trend of P_{sn} to the trends in G_s and T_{pn} under alkaline conditions are unknown, and it is surprising that P_{sn} would reach a maximum under alkaline conditions, while G_s and T_{pn} reached maximum rates under acidic conditions. For this reason it is unlikely that P_{sn} could simply be explained by the quantum yield of photosystem II (ΦPSII) which generally explains decreased photosynthetic rate as being a result of the inhibition of light reactions.

In *Leucadendron salignum* the effect of phosphorus concentration is intensified by pH and this probably explains the steep decrease in PSII under acidic conditions at the highest phosphorus concentration. Under these conditions, P availability is increased due to a greater solubility of P complexes (e.g. $CaHPO_4$). Elevated P may have resulted in a steep decrease in the proportion of light absorbed by chlorophyll (associated with PSII) and light utilised in photochemistry, leading to photoinhibition and decreased P_{sn} rates (Pieters *et al.* 2002). At the other end, P availability decreases in alkaline conditions and this may have resulted in the minimal growth at the lowest P concentration under these conditions. Since both growth and P_{sn} in *Ld. salignum* reached minimum rates at the lowest P concentration it is likely that P deficiency had lead to low sink demand by means of end-product limitation. Previous studies (Pieters *et al.* 2002, Cramer 2006, unpublished) have shown that the demand for sucrose synthesis is decreased due to decreased growth, and this ultimately restricts recycling of P_i to the chloroplasts which limits ATP (adenosine triphosphate) synthesis as well

as RuBP (Rubisco) regeneration. RuBP is the rate limiting enzyme in photosynthesis and therefore P_{sn} rates are affected by the activity of this enzyme.

Leucadendron coniferum have evolved mechanisms such as extremely efficient P uptake via cluster roots under very low soil [P]. However, previous studies on P toxicity in Proteaceae spp. have found that the normal capacity of plants with a low absorption rate (due to low P availability) to store P in leaves are exceeded (e.g. Hakea prostrata, Shane *et al.* 2004a) under unregulated P uptake. This is likely relevant to *Ld. coniferum*, particularly under acidic conditions where P availability is elevated. Abnormal P accumulation in leaves may have resulted in $CaHPO_4$ precipitation in leaf epidermal tissues, thus decreasing the availability of Ca. This cation is extremely important in signalling and partly regulates stomatal conductance, thus may explain the adverse effects of high [P] on P_{sn} in this Proteaceae species.

This study is in agreement with previous work on P deficiency-limited-photosynthesis in a cluster root forming crop species (*Lupinus albus*) in that relatively low concentrations of phosphorus (1 μg P g^{-1} sand) were required for relatively high rates of photosynthesis (Fig. 3 and 5). Furthermore, this species, like in other studies (Cramer *et al.* 2006 unpublished), showed similar responses of P_{sn} and G_s, which shows that G_s was matched to the rates of carboxylation. The relatively steep decrease in P_{sn} between 1 and 10 μg P g^{-1} sand was most likely due to inconsistency in measurements (time of day) rather than a reflection of differences in P_{sn}. However, measurements between 10 and 100 μg P g^{-1} sand were consistent and agree with Cramer et al.'s findings (2006 unpublished) that P_{sn} increments decline as the P concentration increases. Although the reasons in this case are not known, it is likely related to cluster-root formation and activity. The remarkably lower gaseous exchange rates in the non-cluster root forming crop species (*L. angustifolius*) may be a consequence of the inability to mobilise P at low concentrations (due to the lack of cluster roots).

The effect of external P supply and cluster roots on the efficiency of P uptake

Proteaceae species showed an unsaturated increase in P uptake at external supplies of up to 0.2 mmol P m^{-3}, which confirms previous findings in an Australian Proteaceae

species (*Hakea prostrata*, Shane *et al.* 2004b) where rates only decreased for plants grown at 10 mmol P m^{-3}.

Since P uptake did not saturate in this study, Michaelis-Menton kinetic parameters I_{max} and K_m could not be determined. In nutrient poor soils, plants are required to up-regulate uptake by increasing the numbers and activity of high affinity P transporters thereby increasing utilisation of P, however under non native conditions (such as high concentrations of external P), Proteaceae species are unable to down-regulate uptake together with increased mobilisation of insoluble P via cluster root exudates which explains the sensitivity to toxicity in these species (Shane *et al.* 2004b).

The grounds for higher uptake rates in non-cluster roots compared to cluster roots of *Ld. meridianum* are not known, however it would seem that this species might be less dependent on cluster roots for P uptake since it is commonly found on relatively high P soils. This might also explain the small increments in gaseous exchange rates and growth between very low and high P. The relatively lower uptake rates in *Ld. salignum* can be ascribed to the absence of cluster roots, whereas *Ld. coniferum*, a species adapted to very low P, rely greatly on cluster root uptake at levels below 100 µM P (Fig. 4). For all Proteaceae spp., particularly *Ld. coniferum* there is no evolutionary basis for down regulation of uptake, since their natural environments require highly efficient uptake and transport mechanisms, which ultimately leads to phosphorus toxicity in fertilised soils.

Results of this study confirm previous findings (in crop species) of generally faster P uptake rates in cluster roots compared to non cluster roots (Keerthisinghe *et al.* 1998). Uptake rates between the two root types are comparable in early stages but when clusters mature, high numbers of root hairs and mature vascular systems are highly efficient at uptake (Shane *et al.* 2004b). Furthermore, cluster roots can form even under P sufficient conditions and the occurrence of P toxicity in crop species at much higher P concentrations than in Proteaceae species is not surprising. In contrast with Proteaceae, these species have been exposed to fertilised agricultural soils over time and might have evolved the capacity to physiologically down-regulate uptake which explains findings of down-regulation at P supplies of 0.08 mmol P m^{-3} (Shane *et al.* 2004b), whereas Proteaceae are slow growing and therefore require relatively slow P absorption to prevent excessive P accumulation in leaf tissues.

Concluding remarks

Classic P toxicity symptoms were significant in *Leucadendron coniferum*, a cluster root forming Proteaceae species adapted to particularly low phosphorus concentrations, making this species least suitable to post agricultural soils, whereas the limestone species showed greater tolerance to elevated [P]. However these results cannot be generalised because (a) pH was not a significant predictor of P concentration in most cases and (b) to find suitable species for post agricultural fertilised soils would require a substantially larger dataset and a greater range of phosphorus concentrations. Furthermore, greater certainty in terms of causal effects on physiological functions could be achieved by micro-location of elements in leaves. Nonetheless, it was obvious that the Proteaceae species studied did not have the ability to regulate P uptake leading to excess P accumulation in leaf tissues. This not only affects Ca signalling as a result of binding to P, but also the sink/source ratios associated with CO_2 assimilation.

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References

Bond P, Goldblatt P. 1984. Plants of the Cape Flora. J.SA. Bot. 13, (suppl.) 1-455.

Bhatti AS and Loneragen JF. 1970. Phosphorus concentration in wheat leaves in relation to phosphorus toxicity. Agron. J. 62, 288-290.

Diatloff E. and Rengel Z. 2001. Compilation of simple spectrophotometric techniques for the determination of elements in nutrient solutions. *Journal of Plant Nutrition.* 24(1): 75 – 86.

Hawkins H-J, Wolf G and Stock WD. 2005. Cluster roots of *Leucadendron laureolum* (Proteaceae) and *Lupinus albus* (Fabaceae) take up glycine intact: an adaptive strategy to low mineral N in soils? *Ann. Bot.* 97: 1275-1282.

Hinsinger, P. 2001. Bioavailability of Soil Inorganic P in the Rhizosphere as Affected By Root-induced Chemical Changes: A Review. *Plant and Soil* 237: 173- 195

Keerthisinghe G, Hocking P.J, Ryan P.R and Delhaize E. 1998. Effect of phosphorus supply on the formation and function of proteoid roots of white lupin (*Lupinus albus L.*). *Plant, Cell and Environment* 21: 467 – 478.

Marschner H 1995. Mineral Nutrition of Higher Plants, 2nd Edition. Academic Press, London.

Maxwell K and Johnson GN. 2000. Chlorophyll fluorescence- a practical guide. *Journal of Experimantal Botany*. 51(345): 659-668.

Mustart, PJ and Cowling RM. 1993. The Role of Regeneration Stages in the Distibution of Edaphically Restricted Fynbos Proteaceae. *Ecology*, Vol. 74, No. 5: 1490-1499

Mitchell DT, Brown G, Jongens-Roberts SM. 1984. Variation of forms of phosphorus in the sandy soils of the coastal Fynbos, south-western Cape. *J. Ecol.* 72: 575-584.

Pieters AJ, Paul MJ and Lawlor DW. 2001. Low sink demand limits Photosynthesis under Pi deficiency. *Journal of Experimental Botany*

52(358): 1083-1091

Raghothama, KG and Karthikeyan AS. 2005. Phosphate acquisition. *Plant and Soil.* 274: 37- 49

Shane MW, Szota C and Lambers H. 2004b. A root trait accounting for the Extreme phosphorus sensitivity of *Hakea prostrate* (Proteaceae). *Plant, Cell And Environment.* 27: 991 – 1004.

Shane MW, McCully MF and Lambers H .2004a. Tissue and cellular phosphorus storage during development of phosphorus toxicity in *Hakea prostrata* (Proteaceae). *Journal of Experimntal Botany* 55(399): 1033-1044.

Shane MW and Lambers H. 2006. Systemic suppression of cluster-root formation and net P-uptake rates in *Grevillea crithmifolia* at elevated P supply: a Proteacea with resistance for developing symptoms of 'P toxicity'. *Journal of Experimental Botany.* 57(2): 413- 423

Witkowski, E.T.F and Mitchell, D.T. 1987. Variations in Soil Phosphorus in the Fynbos Biome, South Africa. *Journal of Ecology*, Vol. 75: 1159-1171

Appendix A

The effect of phosphorus concentration and pH on growth and biomass allocation

Fig. 1. Phosphorus toxicity symptoms in a cluster root forming crop species a) *L. albus* at level 3 (phosphorus concentration of 100 µg P g sand) and a non cluster root forming species b) *L. angustifolius*

Phosphorus uptake rates at various concentrations of external P supplies and cluster root formation

Fig 2. Cluster root formation in *Ld. coniferum* after a 3 ½ month trial period in P depleted hydroponic solution a) root-shoot; b) root system (cluster and non-cluster) c) cluster roots

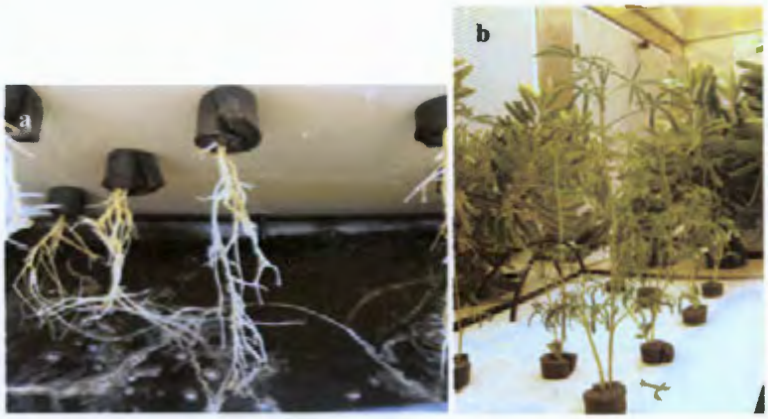

Fig 3. Lupinus angustifolius in hydroponic solution a)non-cluster roots b)shoots

Fig. 4. Root systems of Lupins a) non-cluster root pieces from *L. albus* and b) cluster root pieces from *L. albus* in the tubes used for P uptake kinetics; c) the root system of *Lupinus albus* showing cluster roots; d) the root system of *Lupinus angustifolius* (non cluster root forming)

Table 1. The transformed and untransformed data for *L.albus* cluster root uptake rates at various concentrations of external P supplies.

Log			Untransformed P uptake rates		
Groups [P]	log [P]	log Uptake	[P]	Averages Uptake (pmol g-1 dm s-1)	stderror
1	1.01	2.6154496	10.51	439.7	96.94
1	1.12	3.1547591	13.44	1005.4	959.63
1	1.13	2.7086366	13.59	575.4	167.13
2	1.97		95.32	-7361.3	2104.05
3	2.97	4.4105866	914.91	30617.8	10473.10
3	3.41	5.6287007	2698.87	446275.6	75548.60

Table 2. Transformed phosphorus concentrations for Proteaceae species with the corresponding [P] groupings, untransformed uptake rates and P concentrations.

Species& Root type	Logs		Untranformed data	
	Groups Log [P]	Log [P]	[P]	P Uptake rate averages (n=4)
Ld. coniferum				
Cluster roots				
	1	3.41	19	943.359
	2	4.29	111	2745.53
	3	4.41	1013	5169.34
	3	4.42	1708	9568.77
Non cluster roots				
	1	0.593550136	3.9	119.5709
	1	0.8259762	6.7	248.1227
	2	1.779022804	60.1	1426.511
	3	2.732697256	540.4	4193.547
Ld. salignum				
Non cluster roots				
	0.5	0.21271643	2	1.750512
	1	0.67955399	5	95.16075
	2	1.78773576	61	899.5403
	3	2.8201129	661	3844.573
Ld. meridianum				
Cluster roots				
	0	0.446064	3	2.576623
	1	0.761376	6	297
	2	1.898194	79	3308
	3	2.80006	631	11557
Non cluster roots				
	1	0.6476075	3016.265	4
	1	0.7259116	2219.122	5
	1	1.0199467	2697.64	10
	2	1.7798044	9326.219	60
	2	2.0451077	3731.896	111
	3	2.8813881	13373.56	761
	3	3.0672522	15914.73	1167