Phosphorous uptake rate in two low phosphorous adapted species, *Aspalathus linearis* and *Podalyria calyptrata*

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ABSTRACT

Due to the low P nature of soils within the fynbos biome of the Cape Floristic Region, plants have developed numerous mechanisms which enable them to better acquire phosphorous. A number of species have been reported to have specialised root morphologies (root clusters, mycorrhizae) that enhance P uptake. Plants may also down-regulate the uptake of P by decreasing the expression of genes that encode P transporters. Two Fabaceae species, *Podalyria calyptrata* and *Aspalathus linearis*, were grown in hydroponics for 5 months at a low P supply of 4μM and P-depletion studies were conducted thereafter at 5 levels of external P (4, 10, 20, 50 and 100μM). Growth rates (biomass accumulation) were also calculated as were root to shoot ratios for both species. *A. linearis* had a higher uptake rate than *P. calyptrata*. While the fresh biomass growth rate was similar in both species, *P. calyptrata* had a higher dry weight root to shoot ratio than *A. linearis*. The results showed that both species exhibited a lack of response to increasing P concentrations and had similar RGRs. Their uptake rates differed significantly ($p<0.05$) and this was likely due to their different root:shoot ratios. This indicates that both species would effectively grow in low P soils and in the case of *P. calyptrata*, in high P soils as well.

INTRODUCTION

The South African Cape Floristic Region compromises an area of ca. 90 000km^2, less than 5% of the total area of the southern African continent, yet has been identified as one of the most diverse areas in the world due to its high species richness and endemism not seen anywhere else (Goldblatt and Manning 2002, Linder 2003). The CFR is comprised of five biomes and several distinctive vegetation types with some of the main families of plants that occur in the area being Aizoaceae,

Fabaceae, Proteaceae, Restionaceae and Ericaceae (Goldblatt and Manning 2002, Linder 2003). The CFR is home to some 9000 and more species of vascular plants, of which 69% are endemic (Goldblatt and Manning 2002, Linder 2003). The most common and distinctive biome in the CFR is fynbos which is characterised by the presence of Proteaceae, Restionaceae and Ericaceae (Goldblatt and Manning 2002, Shane *et al.* 2008). This region is made up of a mosaic of soil types which are derived from various parent materials including sandstone, granite, limestone and shale (Goldblatt and Manning 2002). Fynbos typically occurs on sandstone derived soils that are generally poor in nutrients (Shane and Lambers 2005, Ojeda *et al.* 2001, Goldblatt and Manning 2002), highly leached and very deficient in exchangeable bases, total nitrogen (1 to 2mgN g^{-1}) and available phosphorous (0.4 to 3.7μgP g^{-1}) (Cramer 2010, Hawkins *et al.* 2007, Herppich *et al.* 2002, Wisheu *et al.* 2000). Plant available P is low in soils because most of the P is adsorbed to Ca^{2+}, Al^{3+} or Fe^{3+} cations or is in organic form and therefore inaccessible to plants (Gahoonia and Nielson 2004).

Due to the low-P nature of soils within the fynbos biome, plants have developed a number of mechanisms to enable them to better conserve P, such as sclerophylly, serotiny, efficient P remobilization from senescing leaves and roots and more P allocation to seeds (Cramer 2010, Goldblatt and Manning 2002, Hawkins *et al.* 2007, Shane *et al.* 2008). Some plants also have ways in which to acquire P more efficiently such as cluster roots, mycorrhizal symbioses and exudation of carboxylates and phosphatases (Cramer 2010, Shane and Lambers 2005, Shane *et al.* 2008). A number of species of Proteaceae, Restionaceae, Cyperaceae and Fabaceae have been reported to have specialised root morphologies (cluster roots and mycorrhizal symbioses) for enhanced P-uptake which allows them to persist in the low P soils of the CFR (Power *et al.* 2010). Cluster roots release exudates such as carboxylates, phosphatases, phenols and protons that enhance the availability of P from organic and inorganic forms as well as provide an increased surface area for the uptake of mobilized P (Denton *et al.* 2007, Richardson *et al.* 2009, Power *et al.* 2010, Vance 2001). They have a short lifespan and form on lateral roots as closely packed tertiary roots with a dense covering of root hairs (Richardson *et al.* 2009). In families where root clusters are prevalent, such as Proteaceae, mycorrhizal symbioses are uncommon although exceptions have been found (e.g. *Hakea verrucosa*, Boulet and Lambers 2005). In contrast to this, many species of Fabaceae have mycorrhizal symbionts (Allsopp and Stock 1993) and most lack cluster roots (Cramer 2010, Power *et al.* 2010) but there have been reports of cluster roots in genera such as *Cyclopia* (Spriggs and Dakora 2009) and *Aspalathus* (Allsopp and Stock 1993). The utility of *Aspalathus* species to employ both cluster roots and mycorrhizal symbiosis might seem beneficial in terms of maximizing

P-uptake, however it might be compromised by the high metabolic costs of cluster roots (Shane and Lambers 2005) and mycorrhizae (Lambers *et al.* 2002). In addition to the adaptations and specializations plant roots exhibit, they may also down-regulate the uptake of P (Raghothama 1999) by decreasing the expression of genes that encode P transporters (Smith *et al.* 2003). P is absorbed via the roots from the soil by a combination of two systems, a constitutive low-affinity P-uptake system and an inducible, energy-driven, high-affinity P-uptake system that is regulated by the availability of P (Smith *et al.* 2003, Raghothama 1999). Plants that are supplied suboptimal P concentrations increase their rate of P uptake per unit root mass by up-regulating the numbers and activity of the high-affinity P transport molecules (Raghothama 1999). Conversely, at higher P concentrations supplied to plants, P uptake rates are decreased by down-regulation of numbers and activity of transporter molecules (Smith *et al.* 2003, Raghothama 1999).

P-depletion studies can determine the rate at which plants deplete P from an external solution (Claasen and Barber 1974). Slow-growing plants that are adapted to infertile soils are expected to have a low P-absorption capacity because diffusion to the root surface is the major rate-limiting step in low-P soils and cannot be overcome by an increased capacity of absorption (Nye 1977). In addition, the level of P demand to support plant growth is the major determinant of P-absorption rates (Clarkson 1985). P-uptake kinetic parameters; V_{max}, maximum influx, K_m, the Michaelis constant which is concentration when influx is half of V_{max}, and C_{min}, which is concentration when influx is zero, can be estimated using a Michaelis-Menten model (Brix *et al.* 2010, Claassen and Barber 1974, Lee 1982). These parameters show the relationship between external P concentrations and uptake rates of plants (Brix *et al.* 2010, Claassen and Barber 1974, Lee 1982).

This study aims to determine whether *Aspalathus linearis* and *Podalyria calyptrata,* two indigenous CFR legume species, have similar P-uptake rates. *Aspalathus linearis* (rooibos tea) is restricted to the Cedarberg Mountains, in extremely nutrient poor acidic soils and has the ability to produce nitrogen-fixing nodules, cluster roots and mycorrhizal symbioses (Govender 2007, Sprent *et al.* 2010, Muofhe and Dakora 1999). *Podalyria calyptrata* (water blossom-pea) also nodulates and is found on the ravines and riparian vegetation in the SW CFR (Notten 2004, Schutte-Vlok and van Wyk 2011). It can exhibit re-sprouting and reseeding strategies (Schutte-Vlok and van Wyk 2011). Previous studies have shown that *A. linearis* is less responsive to P fertilization than *P. calyptrata* and it is therefore hypothesized that *A. linearis* will have a higher uptake rate than *P. calyptrata* at low P.

MATERIALS AND METHODS

Plant Growth

Seeds of rooibos (*Aspalathus linearis*) and water blossom-pea (*Podalyria calyptrata*) were germinated in moist sand. After 30 days, 60 seedlings of uniform size (for each species) were selected, and washed free of sand. Then 2 seedlings were transferred to a 5L black plastic pot containing aerated distilled water and seedlings were inoculated with *rhizobia* by applying 1ml of *rhizobia* culture to each black pot. Shoots were gently supported in circular holes made in plastic lids by grey foam discs that acted as tight seals without damaging the plant stem or roots. For the first two days, plants were kept in distilled water solutions thereafter each pot contained 5L of continuously aerated nutrient solution (pH 6.5) of the following composition (in µM): 401 NO_3^-, 201 Ca^{2+}, 200 K^+, 154 SO_4^{2-}, 54 Mg^{2+}, 10.12 Fe-EDTA, 0.24 Mn^{2+}, 0.102 Zn^{2+}, 0.018 Cu^{2+}, 2.4 H_3BO_3, 0.03 Mo^{4+}. The basal solution contained 4µM P as KH_2PO_4. Pots were thinned to one plant after 30 days of growth in hydroponics. Plants were left to grow for 120 days in a glasshouse and nutrient solutions were replaced weekly.

Growth Rates and Root:Shoot Ratios

A set of ten random *P. calyptrata* and *A. linearis* plants were chosen for biomass growth rate determination. The ten plants were weighed 30 days after thinning and again at harvesting for total fresh biomass. The Relative Growth Rate (g g^{-1} d^{-1}) was calculated according to Khandan-Mirkohi and Schenk (2009):

$$\mathrm{RGR} = \frac{\ln(\mathrm{FW}_2) - \ln(\mathrm{FW}_1)}{t_2 - t_1}$$

where *FW* describes plant fresh weight (g $plant^{-1}$), *t* is time (day); subscripts 1 an 2 refer to the first and second measurement, respectively. Root:shoot ratio was determined by the following equation:

$$\text{Root: Shoot ratio} = \frac{\mathrm{RDW}}{\mathrm{SDW}}$$

where *RDW* and *SDW* are root dry weight and shoot dry weight respectively.

Net P-uptake rates determined for whole root systems

Net P-uptake rates were determined for whole root systems of *P. calyptrata* and A. *linearis* by P-depletion studies from the external solution. Twenty-five plants of similar size were chosen for each species and they were transferred into phytotron chambers set at the following conditions: 14hrs daylight, 10hrs night-time, temperature at constant 25°C. The day before the P-depletion measurements, plants were transferred into basal nutrient solution with zero P. From preliminary uptake experiments it was determined that to obtain a linear depletion of P, volumes of 2L for *A. linearis* and 5L for *P. calyptrata* were required. The morning of the experiment, plants were transferred into pots containing half concentration of basal nutrient solution minus P. After 3hrs, the nutrient solution was adjusted to contain P levels of 4, 10, 20, 50 and 100μM for the depletion measurements. Each P level was replicated 5 times. After 1min of P level adjustment in the aeration pot, a 1 mL sample was taken for the determination of total P at time zero and subsequent samples were taken every 30min over a period of 4.5h. Total P concentrations in the 1ml samples were determined using the malachite green colorimetric method (Motomizu *et al.* 1983). Plants were immediately harvested, weighed for FW for growth rate determination, and separated into roots and shoots (leaves and stems). Samples were weighed again after drying at 70°C for 3 days.

The rate of P depletion (μM P h^{-1}) from the external solutions was calculated from linear slopes over a 4.5h period. Net P-uptake rates were calculated using the following equation:

$$\text{nmol P g}^{-1}\text{ root dry weight (DW)s}^{-1} = \frac{\left(\text{PD} * \text{PV} * \left(\frac{1}{(60 * 60)}\right) * 1000\right)}{\text{RDW}}$$

where *PD* is rate of P depletion, *PV* is the volume of the pot used and *RDW* is root dry weight.

Statistics

Growth rate and root:shoot data were analysed for significant differences between the two species using a t-test at $p < 0.05$. Uptake rates were analysed for significant differences between [P] and species using a Two-Way ANOVA at $p < 0.05$ (Statistica; Tulsa, USA).

RESULTS

Comparison of growth rates and root:shoot ratios

The growth rates of *P. calyptrata* and *A. linearis* were similar with rates of biomass accumulation of 0.0208g g^{-1} d^{-1} and 0.0182g g^{-1} d^{-1} respectively (t_s=2.04, df=14, $p>0.05$) (Fig. 1). The root to shoot ratio of 0.57 in *P. calyptrata* was ca. 2-fold greater than *A. linearis* (t_s=-11.25, df=47, $p<0.01$) of 0.33, indicating also that *P. calyptrata* had more roots than shoots in overall biomass while for *A. linearis* had more shoot than root (Fig. 2).

Net P-uptake by whole root systems of intact plants

The strength of the response of the net P-uptake rates to external P concentrations varied between the two species. The response of net P-uptake rates by *A. linearis* show a steep decline in net P-uptake rates that were correlated to increasing external P concentrations (Fig. 3). However the average net P-uptake rates for each external P level for *A. linearis* were not significantly different between each other. The response of net P-uptake rates by *P. calyptrata* showed a similar trend however there was an initial increase of P-uptake followed by a gradual decline correlated to increasing external [P] (Fig. 3). Uptake rates were significantly different between species ($F_{1,3}$=9.78, $p < 0.05$). Maximum P-uptake for *A. linearis* was 2.6nmoles P g^{-1} root DW s^{-1} which occurred at 4µM external P and declined at increasing levels of external P (Fig. 3) while maximum P-uptake for *P. calyptrata* was 1.4nmoles P g^{-1} root DW s^{-1} which occurred at 20µM of external P (Fig. 3).

DISCUSSION

The influence of external P concentrations on net P-uptake rates for two legume species was assessed by growing them at low P levels (4 µmolar P) due to the predominantly low P CFR soils. The initial objective of this research was to obtain V_{max} and K_m values for *A. linearis* and *P. calyptrata* using Michaelis-Menten kinetics though this was not possible due to both species

exhibiting a steep decline in uptake rates which is not characteristic of Michaelis-Menten kinetics (Fig. 3). However, the uptake rates for the two species could be interpreted.

The results showed that *A. linearis* has greater net P uptake rates than *P. calyptrata*. In addition, *A. linearis* showed a maximum net p-uptake rate at 4µM external compared to a maximum net p-uptake rate at 20µM external P for *P. calyptrata* (Fig. 3) which is consistent with the hypothesis.

The two species were grown in the same environment, for the same length of time and exhibited no significant differences in their relative growth rates (Fig. 1). However their root:shoot ratios were significantly different (Fig. 2) and showed that total plant biomass of *P. calyptrata* and *A. linearis* were 57% and 33% root mass respectively. Since the RGRs were not significant, the net P uptake rates are therefore different likely due to the root:shoot ratios of the two species. In previous studies (Brix *et al.* 2010, Rubio *et al.* 1997, Lambers *et al.* 2006) it was reported that plants that produce less root biomass had greater P-uptake rates and that higher root:shoot ratios suggest a reduced capacity to uptake nutrients (Power *et al.* 2010). Plants increase their root to shoot ratio when P is limiting which increases the surface area volume for absorption therefore plants need not have high uptake rates as the increased root biomass compensates for the low uptake rate (Gahoonia and Nielsen 2004, Lee 1982, Raghothama 1999, Shane *et al.* 2004, Shane and Lambers 2005, Shane *et al.* 2008, Brix *et al.* 2010).

Another difference noticed during the experiment was that *A. linearis* produced simple cluster roots (Fig. 4), while *P. calyptrata* produced dauciform-like roots (Fig. 5), which are similar in function to cluster roots (Shane *et al.* 2006). Thus the presence of these specialised root formations in both plants indicates that P was deficient in the system as both root types are suppressed when there is sufficient P (Lambers *et al.* 2006).

Since the distribution of *A. linearis* in the CFR shows that it is predominantly grown in the sandy Clanwilliam areas that have lower P soils than the *P. calyptata* habitats of the south western Cape soils (Govender 2007, Sprent *et al.* 2010, Muofhe and Dakora 1999, Notten 2004, Schutte-Vlok and van Wyk 2011), it is therefore expected that *A. linearis* have a higher net P uptake rate than *P. calyptrata*. These observations are consistent with a number of studies that showed that at low P supply, plants exhibit a high P uptake rate which is due to up-regulation of high affinity P transporters (Lambers *et al.* 2006, Brix *et al.* 2010) associated with efficient P uptake kinetics (Lee 1982; Jungk *et al.* 1990; Bhadoria *et al.* 2004, Shane *et al.* 2004). However, these results contradict the report of Shane *et al.* (2008) where *Protea compacta* growing in low P colluvial sands showed

lower net P uptake rates than those of *P. obtusifolia* and *Leucadendron meridianum* found in high P limestone soils.

The general lack of response in P uptake of both *P. calyptrata* and *A. linearis* to increasing P supply is effective in the naturally low P environments where the plant may never be exposed to high P. *A .linearis* is adapted to living in naturally low P soils and therefore the P uptake rate is non-responsive at higher P concentrations. *P. calyptrata* however naturally occurs in soils where P is more abundant and therefore should have had a greater response to the increasing external P concentrations however due to it being grown at a lower level of P it exhibited a more plastic uptake system than did *A. linearis* therefore allowing it to take up P over a wide range of external P concentrations and promote growth. *P. calyptrata* also had a significantly lower uptake rate compared to *A. linearis* and this is likely due to its significantly different root:shoot ratio therefore allowing it to take up more P per surface area volume.

In conclusion, it was found that both plants lacked a response to increased external P concentrations however they exhibited different uptake rates likely due to their differing root:shoot ratios. Both species would effectively grow in low P soils and in the case of *P. calyptrata*, in high P soils as well. Habitat specificity plays a huge role in maintaining high biodiversity in South Africa (Goldblatt and Manning 2002, Linder 2003) and root adaptations and traits for P acquisition may contribute to these high levels of biodiversity.

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Figures

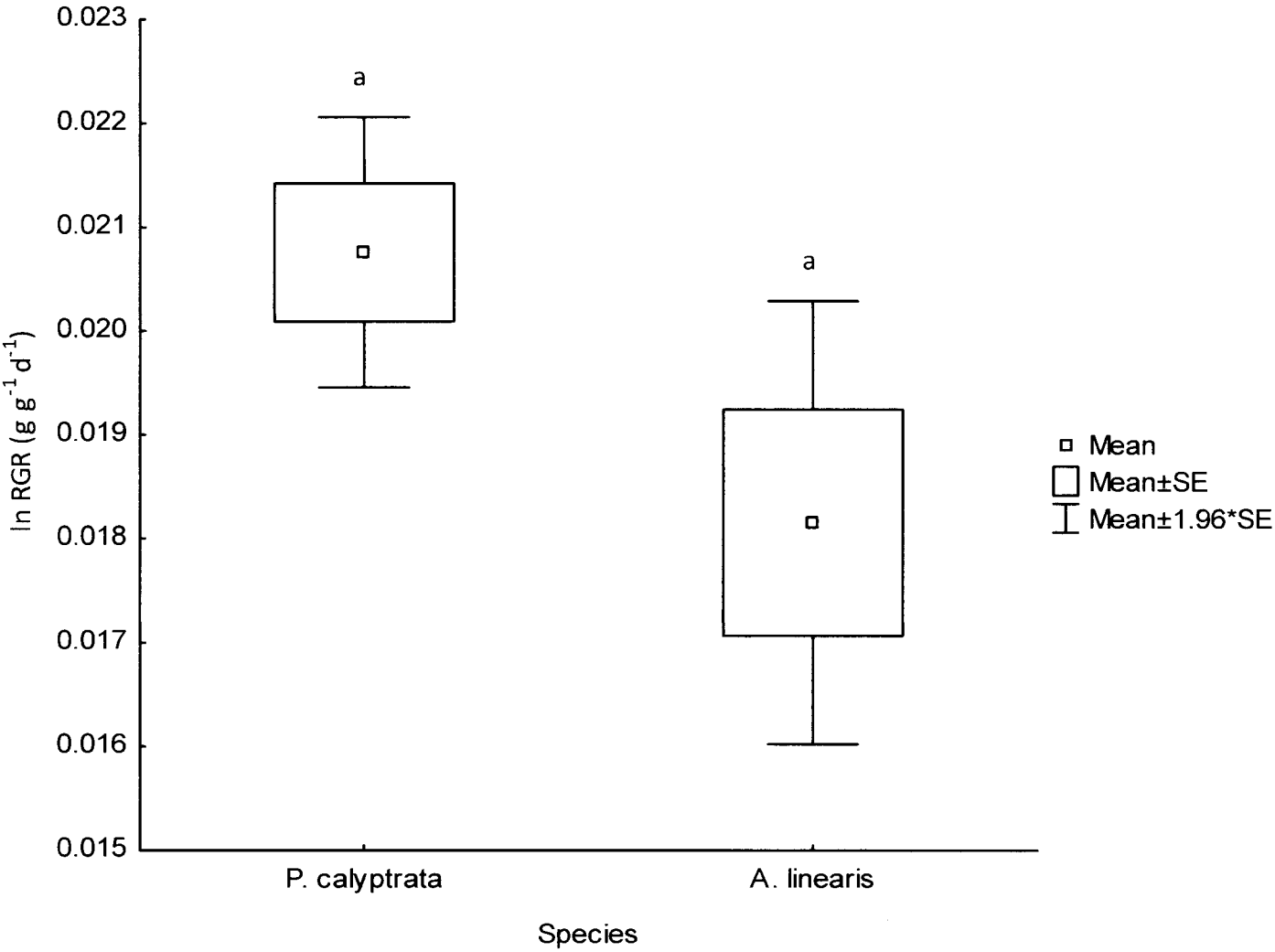

Figure 1: Relative growth rates in g g^{-1} d^{-1} for *P. calyptrata* and *A. linearis*

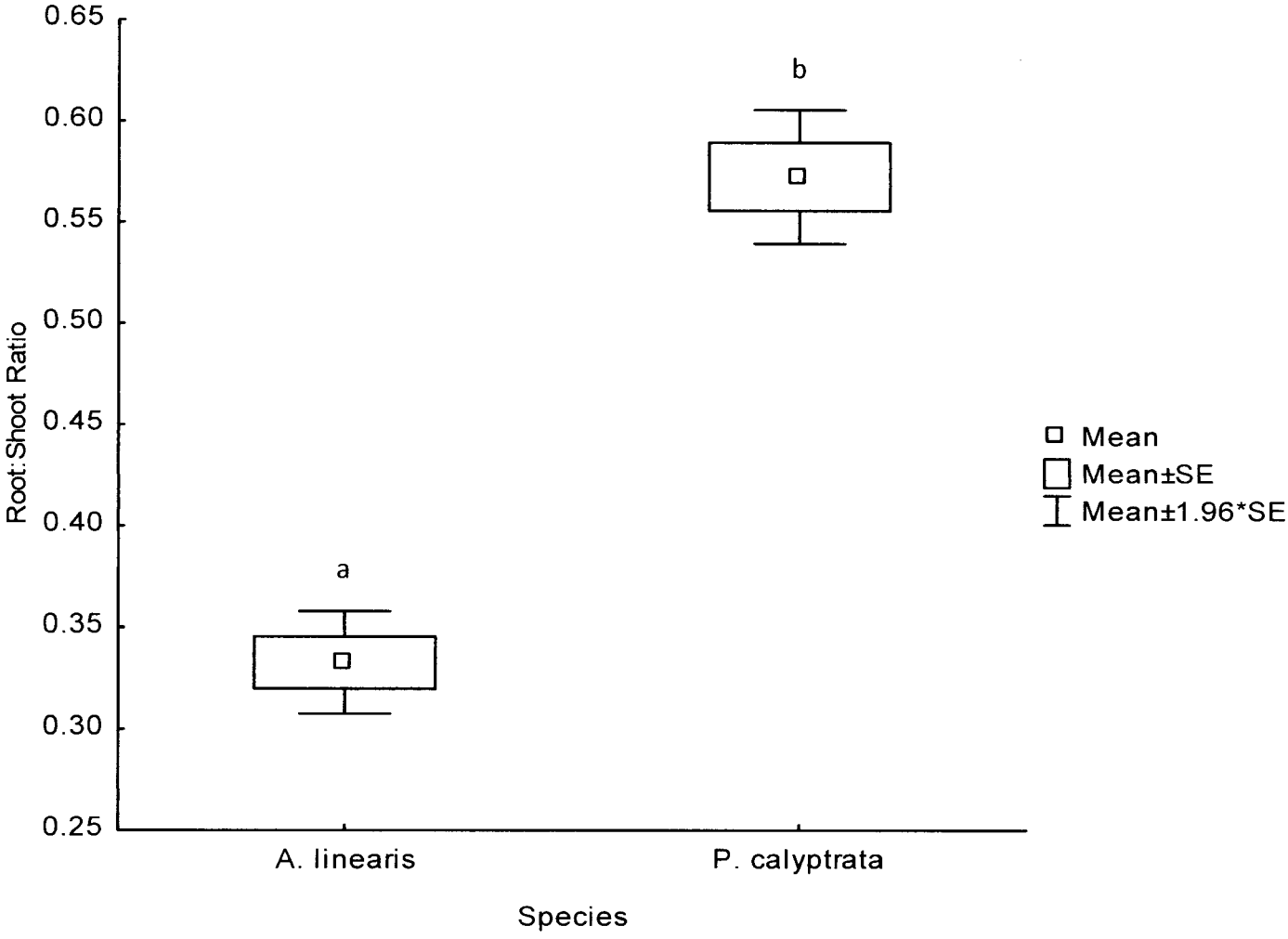

Figure 2: Average root:shoot ratios for *P. calyptrata* and *A. linearis*

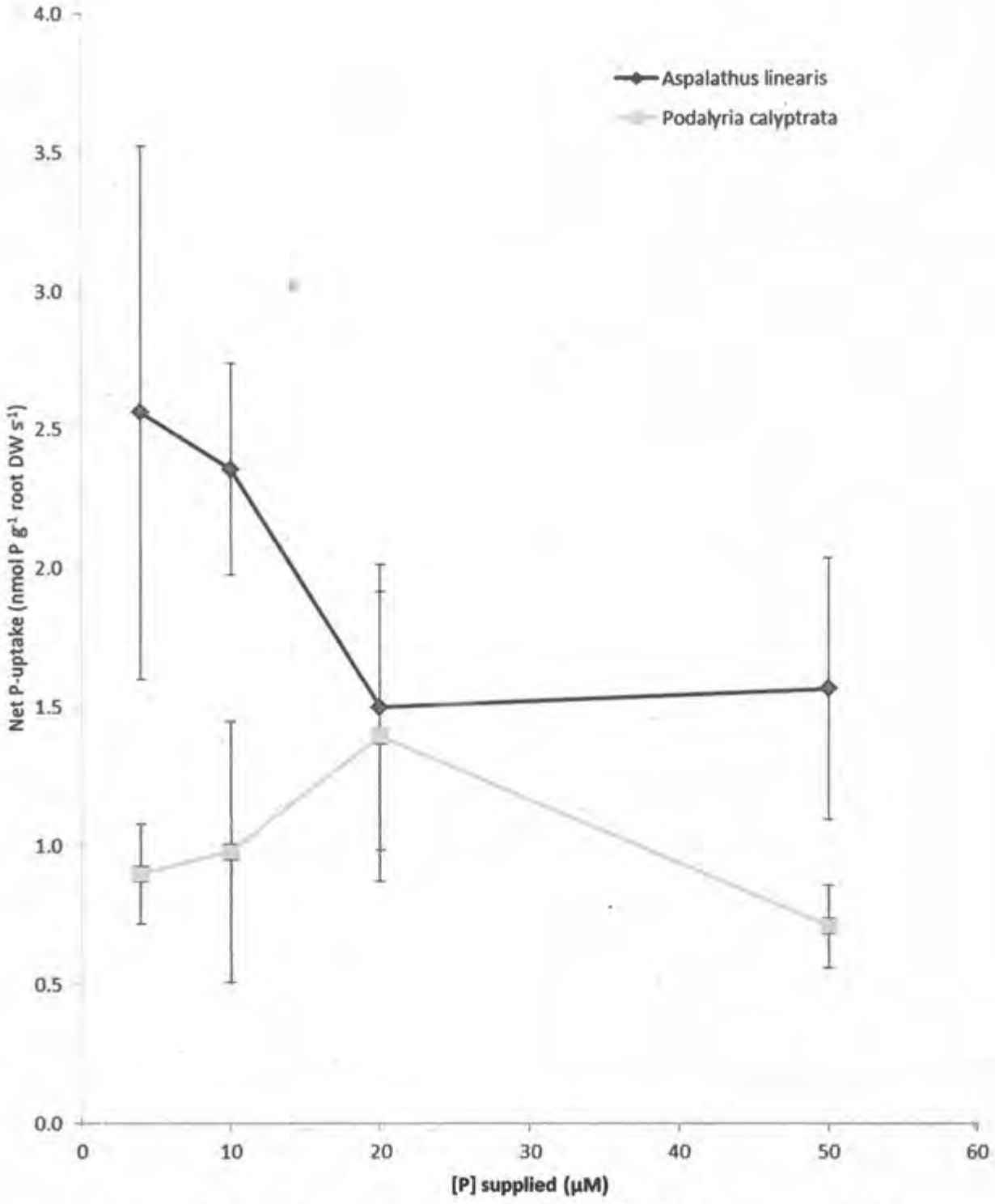

Figure 3: Net P-uptake rates (nmol P g^{-1} root DW s^{-1}) of *P. calyptrata* and *A. linearis*. Bars are standard errors (n=5). Net P uptake rate at 100 uM P was not included because negative uptake rates were recorded with large error bars.

Figure 4: Root system of *A. linearis*. (A) Shows a whole root, (B) is a close-up of a clusteroot forming off the lateral root system and (C) is a close-up of a young cluster root developing.

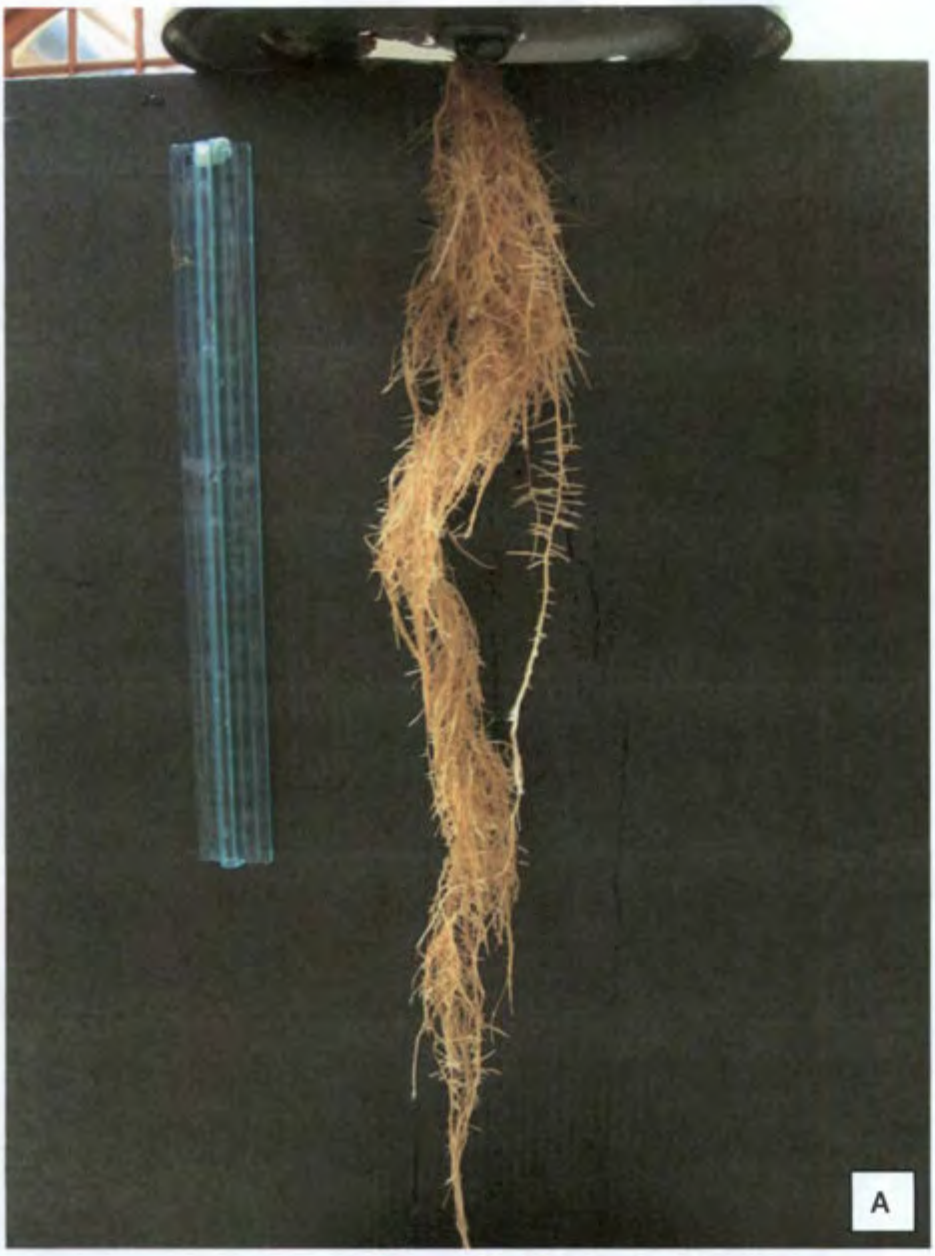

Figure 5: Root system of P. calyptrata. (A) shows a whole root and (B) shows a close-up of the dauciform roots forming off the lateral roots

APPENDIX

Table 1: Root and shoot fresh weights and dry weights for *P. calyptrata* and *A. linearis*

Podalyria calyptrata					*Aspalathus linearis*				
Plant #	Root FW (g)	Root DW (g)	Shoot FW (g)	Shoot DW (g)	Plant #	Root FW (g)	Root DW (g)	Shoot FW (g)	Shoot DW (g)
1	15.92	2.1426	14.66	3.7454	1	2.12	0.1904	3.19	0.6336
2	13.04	2.0651	18.15	4.1592	2	5.78	0.3853	7.12	1.3687
3	13.14	1.8295	14.93	3.4292	3	1.43	0.1319	2.23	0.4211
4	17.12	2.4519	15.90	3.4149	4	1.32	0.1052	2.11	0.4195
5	12.80	1.7973	10.10	2.7525	5	1.89	0.1605	3.65	0.6770
6	16.25	2.2202	13.17	3.4892	6	2.38	0.2689	4.31	0.7537
7	16.74	2.4284	24.98	5.6104	7	1.40	0.1432	2.00	0.4210
8	11.30	1.5441	13.39	3.0376	8	2.47	0.2357	3.16	0.5750
9	16.89	2.3054	14.32	3.2819	9	2.20	0.1998	2.94	0.5475
10	11.78	1.4641	12.76	2.4971	10	1.89	0.1562	2.85	0.5571
11	12.49	1.8084	12.09	3.4174	11	2.67	0.2670	3.39	0.5917
12	22.04	2.6672	17.96	4.2043	12	2.12	0.1723	2.17	0.4016
13	16.18	2.4825	16.80	3.6300	13	2.96	0.2628	3.74	0.6960
14	10.29	1.6769	15.77	4.5032	14	2.84	0.2675	3.40	0.6304
15	16.44	2.2849	16.02	4.0080	15	2.17	0.2057	2.39	0.4907
16	15.17	2.0211	14.34	3.4440	16	1.28	0.1284	1.59	0.3931
17	13.19	1.9006	14.87	3.3556	17	3.47	0.2754	4.64	0.8227
18	12.34	1.8152	12.80	3.0727	18	1.02	0.0838	1.74	0.3721
19	24.52	2.3680	19.78	4.3860	19	1.76	0.1598	2.99	0.5569
20	17.43	2.2630	18.65	4.2443	20	1.25	0.1073	1.74	0.4014
21	18.79	2.6187	20.94	5.1840	21	2.28	0.1785	3.10	0.5545
22	17.39	2.8260	12.80	3.9757	22	3.01	0.2721	4.11	0.7683
23	15.24	2.0487	15.93	3.4289	23	1.90	0.1792	2.66	0.5454
24	15.32	1.7894	16.44	3.5718	24	3.72	0.3172	5.61	1.0398
25	14.39	1.9151	15.56	3.4482					

Table 2: Uptake rates (nmol P g^{-1} root DW s^{-1}) for all replicates of *A. linearis* and *P. calyptrata* at increasing external P concentrations over 4.5h.

	P Concentration (µM)									
	Aspalathus linearis					*Podalyria calyptrata*				
Minutes	4	10	20	50	100	4	10	20	50	100
0	5.660	14.653	25.134	40.591	46.037	9.211	11.004	22.983	42.582	47.999
30	6.119	13.436	20.842	42.325	44.831	10.385	10.687	19.419	40.198	45.525
60	24.133	11.509	22.724	44.981	51.838	7.901	25.886	22.580	39.793	48.630
90	4.893	11.785	20.024	41.627	51.682	7.677	11.941	19.837	38.375	49.352
120	4.953	16.470	26.342	55.110	57.267	6.205	13.387	20.501	48.222	52.475
150	4.824	11.363	20.905	43.492	52.082	5.427	6.582	17.818	40.997	44.970
180	4.265	11.277	22.552	42.158	49.763	5.761	9.434	19.661	39.777	46.022
270	3.082	8.567	19.439	36.777	51.081	0.110	4.317	17.562	38.957	47.707
0	5.660	14.653	23.752	40.591	46.037	9.211	11.004	23.917	42.582	47.999
30	4.664	12.993	22.141	45.175	52.477	6.321	11.393	25.819	41.135	44.161
60	7.734	15.644	22.200	45.272	50.071	8.579	12.518	20.873	42.268	46.786
90	4.640	14.567	22.359	47.574	52.932	6.978	11.159	22.140	41.966	47.870
120	6.405	16.869	24.378	54.149	50.508	4.806	12.772	21.440	43.241	53.033
150	5.900	14.304	21.085	42.254	53.420	5.245	11.715	21.176	38.354	48.362
180	5.213	13.808	21.203	40.168	46.565	3.106	9.464	19.712	40.902	50.368
270	3.391	10.564	20.493	40.943	40.592	2.872	9.157	16.893	37.748	45.941
0	6.891	14.653	23.752	40.591	46.037	9.211	11.004	22.983	43.302	50.286
30	5.878	14.548	22.020	51.850	47.703	5.990	8.174	21.178	42.139	47.396
60	5.754	12.064	23.171	42.727	53.078	8.058	12.360	22.721	48.225	50.299
90	3.498	14.501	22.965	41.472	58.091	6.411	10.976	21.476	43.949	45.886
120	14.177	11.885	25.466	53.322	58.691	4.950	39.004	20.773	43.374	50.589
150	3.391	9.519	21.233	40.770	44.686	3.892	10.240	18.761	41.416	46.889
180	4.233	10.932	22.598	42.001	52.714	3.155	10.705	19.164	41.433	47.935
270	4.388	10.178	22.693	40.626	51.172	1.212	8.595	16.346	40.318	47.715
0	6.891	14.653	25.134	44.149	46.251	8.041	11.746	22.983	43.302	47.999
30	2.854	10.908	23.494	52.755	45.699	3.359	9.210	18.654	49.830	45.527
60	34.833	11.835	22.614	46.516	54.755	4.789	10.121	21.893	68.568	50.866
90	3.839	11.435	20.865	42.774	51.089	5.511	8.978	19.376	43.462	48.251
120	5.633	15.341	23.382	49.032	61.247	6.518	9.033	22.776	41.251	53.767
150	3.482	12.192	23.348	45.695	50.325	2.707	6.987	19.422	67.001	46.292
180	4.443	11.058	20.859	47.900	49.370	4.951	6.470	18.960	42.027	49.372
270	0.438	10.140	24.082	41.308	56.787	3.495	9.236	16.844	41.503	45.190
0	5.660	14.653		40.591	46.251	8.041	11.746	23.917	42.582	47.999
30	3.594	14.461		43.063	67.654	4.647	11.200	29.799	40.295	48.818
60	5.351	12.969		40.375	51.176	4.427	51.935	64.020	41.657	49.510
90	5.219	15.159		46.154	50.847	5.826	10.178	20.386	39.328	47.773
120	9.999	21.697		44.901	58.738	2.878	14.985	27.797	39.743	53.578
150	6.776	11.606		42.652	48.262	3.109	9.276	18.208	36.042	46.717
180	4.012	13.764		41.654	50.640	3.252	8.161	22.993	41.406	46.278
270	3.505	11.414		41.523	44.690	2.757	6.497	16.097	37.121	47.592