Understanding differences in critical external phosphorus requirements of pasture legumes

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Key findings

» *Ornithopus sativus* (French serradella) and *O. compressus* (yellow serradella) had a significantly high root hair cylinder volume compared with *Trifolium subterraneum*, which provides for a great phosphorus (P) foraging ability and helps explain why the *Ornithopus* species has lower critical P requirements than *T. subterraneum*.

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Introduction

This research identified the plant traits most likely to determine differences in external critical phosphorus (P) requirements (i.e. the soil extractable-P concentration required to achieve 90% of maximum yield) of pasture legume species. Understanding trait differences that affect critical P requirements of pasture legumes is important in providing confidence around field differences in external critical P requirement.

Experiment

A glasshouse experiment was established to identify the traits influencing the external critical P requirement of pasture legumes. Twelve pasture legumes (Table 1) were sown at a rate of 50 mg of viable seed per pot. After seven days, pots were inoculated with a slurry of peat containing an appropriate rhizobium strain and placed in a temperature-controlled glasshouse (maximum 20 °C, minimum 16 °C) under natural light (July–August, Wagga Wagga, NSW, Australia).

PVC pots (90 mm diameter; 210 mm height) were filled with 1.2 kg of a low P soil (6 mg/kg Colwell P) with no applied P (subsoil 50–200 mm depth). An additional 403 g of P-amended (topsoil 0–50 mm depth) was then added to the top of each pot. Five replicates of each P rate (0, 15, 30, 60, 90, 140 and 250 mg/kg) were prepared for each pasture legume species. This was used to mimic the stratification of P that occurs with surface fertiliser application on pastures.

Pots were maintained at approximately 80% field capacity by watering to weight three times a week. Plants were harvested six weeks after sowing. Shoots were cut at soil level. Soil was removed from the pot as an intact core and cut at the interface of the P-amended topsoil (0–48 mm height) and un-amended subsoil.

Table 1. Rhizobium inoculant group and strain for different pasture species.

Scientific name	Common name	Cultivar	Rhizobium inoculant group and strain
Ornithopus compressus L.	yellow serradella	Santorini	Group S, WSM471
Ornithopus sativus Brot.	French serradella	Margurita	Group S, WSM471
Biserrula pelecinus L.	biserrula	Casbah	Biserrula special, WSM1497
Trifolium michelianum Savi.	balansa clover	Bolta	Group C, WSM1325
Trifolium vesiculosum Savi.	arrowleaf clover	Zulu II	Group C, WSM1325
Trifolium glanduliferum Boiss.	gland clover	Prima	Group C, WSM1325
Trifolium hirtum All.	rose clover	Hykon	Group C, WSM1325
Trifolium purpureum Loisel.	purple clover	Electra	Group C, WSM1325
Medicago truncatula Gaertn.	barrel medic	Sultan-SU	Group AM, WSM1115
Trifolium incarnatum L.	crimson clover	Dixie	Group C, WSM1325
Trifolium spumosum L.	bladder clover	Bartolo	Group C, WSM1325
Trifolium subterraneum L.	subterranean clover	Leura	Group C, WSM1325

Roots were washed from each section of the topsoil core, and the entire core of subsoil. Roots from one of the quarters of the topsoil core were immediately scanned using a flatbed scanner and analysed for root length and average root diameter using WinRHIZO (Regent Instruments Inc., Quebec, Canada). Using these roots, two images of fully elongated root hairs were taken using a Nikon SMZ25 stereomicroscope fitted with a high resolution DS-Fi2 digital camera and digital sight DS-U3 camera controller.

Shoots and roots were dried at 70 °C before being weighed for dry mass. Root dry mass from the quarter of topsoil that was scanned was used to calculate specific root length (length per unit dry mass). The total length of roots in the topsoil was then calculated based on the total dry mass measured in the topsoil and the specific root length of the scanned quarter. Root hair length was determined by measuring the length of 10 root hairs (five per image) using the software package ImageJ (Rasband 1997–2014).

Results

Shoot dry matter

Maximum shoot dry matter ranged from 1.75 g/pot (*Biserrula pelecinus*) to 2.53 g/pot (*Medicago truncatula*) (1.4-fold) while critical external requirements for P ranged from 57 (*Ornithopus sativus*) to 203 mg applied P/kg soil (*Trifolium subterraneum*) (3.6-fold, Figure 1). Lower critical external requirements for P were not associated with a lower maximum yield. The *Ornithopus* spp. had significantly lower critical external requirements for P than all other species (57–63 mg applied P/kg soil).

Root dry matter

Topsoil root dry matter for most species increased in response to declining P. However, species generally reached a low-P threshold below which root dry matter was not maintained. Both the peak in topsoil root dry matter and the rate of P application at which it occurred varied between species. *Trifolium subterraneum* and *T. versiculosum* had the most prominent peaks in topsoil root dry matter (0.52–0.47 g) and these peaks occurred at a higher rate of P application (90 mg P applied/kg) than that observed for any other species.

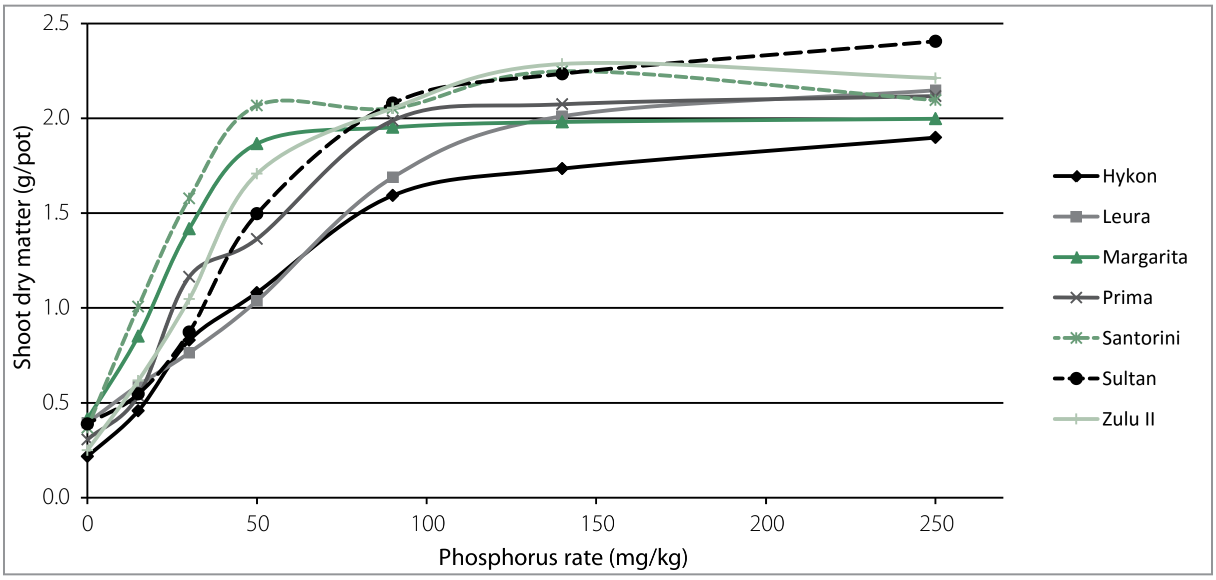

Figure 1. Shoot dry matter in response to P applied for seven of the 12 legume species. l.s.d. = 0.05 g for species × P applied ($P < 0.05$; n = 5).

Root hair cylinder volume (RHCV)

RHCV of the species increased between 2.0 (*T. purpureum*) and 4.3-fold (*O. compressus*) in response to low P supply (figures 2 and 3). The *Ornithopus* spp. and *B. pelecinus* achieved the largest root hair cylinder volumes (136–168 cm^3), which represented approximately 50% of the volume of the topsoil. These maximums occurred at very low levels of applied P relative to most other species (15–30 mg P applied kg). *Trifolium subterraneum* consistently had amongst the lowest root hair cylinder volumes (up to 41.7 cm^3), which represented less than 15% exploration of the volume of the topsoil. Amongst the species, the specific surface area of the root hair cylinder (i.e. surface area of root hair cylinder per unit root dry mass) was negatively correlated with the critical external P requirement. The relationship was generally strongest at lower levels of applied P (e.g. at 15 mg/kg R^2 = 0.87).

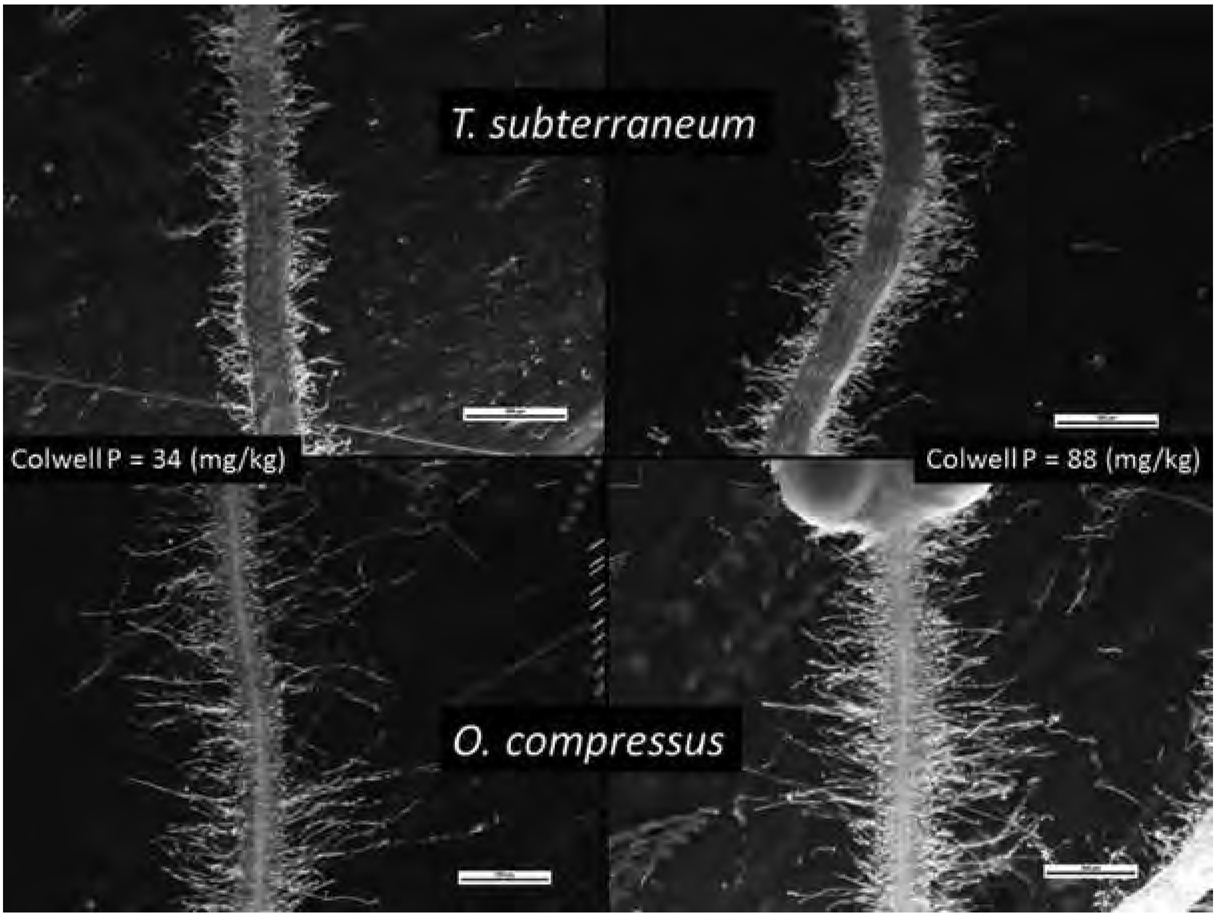

Figure 2. Root hairs of *T. subterraneum* cv. Leura (top half) and *O. compressus* cv Santorini (bottom half) at Colwell P levels of 34 (left hand side) and 88 mg/kg (right hand side). Scale bar: 500 micrometres.

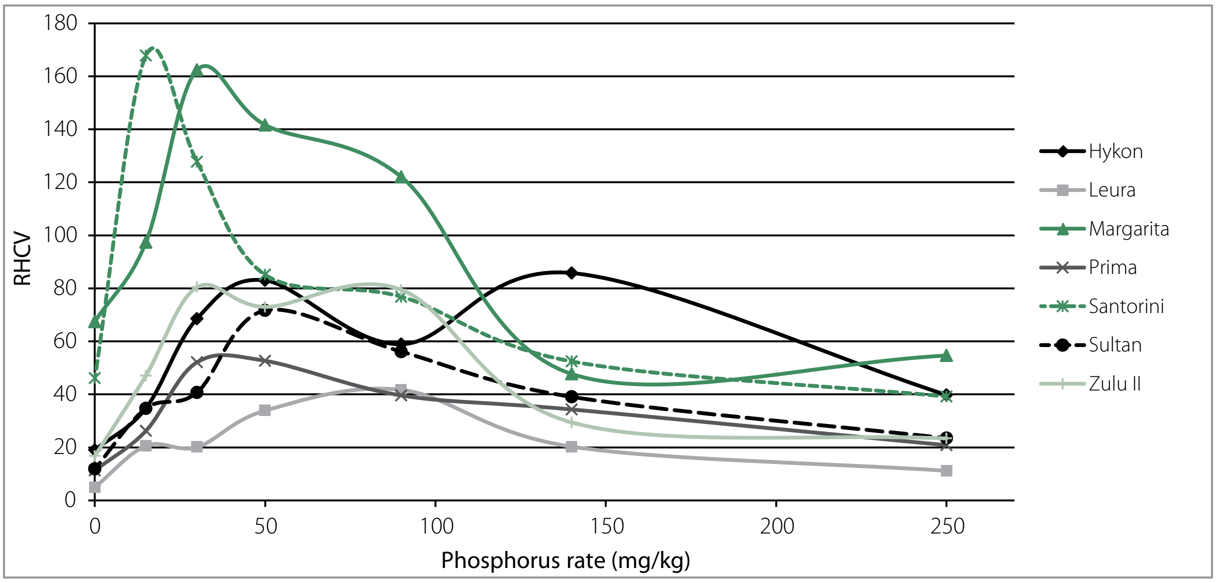

Figure 3. Root hair cylinder volume (RHCV) in response to P applied for seven of the 12 legume species. l.s.d. = 0.05 for species × P applied (P <0.05; n = 5).

Discussion

Long roots combined with long root hairs resulted in a large RHCV, which proved to be an efficient strategy for acquiring P and maximising shoot growth in lower P soils. Among the wider range of species in the present experiment, the *Ornithopus* species demonstrated the greatest ability to achieve a large root hair cylinder. Consistent with findings by Yang et al. (2015) and Haling et al. (2016), *T. subterraneum* ranked amongst the poorest species for nutrient foraging potential because of its very short root hairs.

Conclusion

The results indicate that the *Ornithopus* species has a greater P foraging ability than *T. subterraneum*, which is likely to explain the lower external critical P requirement of *Ornithopus* species. This is further supported by data showing that internal P efficiencies were similar for all legume species. The very low ranking of *Ornithopus* species critical P requirements relative to *T. subterraneum* suggests that pastures based on the *Ornithopus* spp. could deliver an approximately one third reduction in P fertiliser inputs.

References

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Acknowledgements

Meat and Livestock Australia, along with Australian Wool Innovation, NSW DPI, CSIRO and UWA financially supported this research through the project 'Phosphorus efficient legume pasture systems' (B.PUE.0104). Results presented here are a small component of a larger research effort supported by the above organisations.