Phosphorus efficient pastures: variation in mycorrhizal colonisation of subterranean clover

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Abstract
Trifolium subterraneum (subterranean clover) is an important annual pasture legume in southern Australia. Development of more phosphorus (P)-efficient cultivars could improve the P balance of pastures. Subterranean clover hosts arbuscular mycorrhizal fungi (AMF). In glasshouse studies, AMF can enhance P uptake by subterranean clover. To examine whether subterranean clover cultivars differ in their levels of colonisation by AMF, a core collection of subterranean clover (97 lines, representing ~80% of genetic diversity in the species), and 42 cultivars were grown in a glasshouse in a low-P sandy soil with indigenous AMF. The soil was free of root diseases. The percentage of root length colonised by AMF, specific root length, average root diameter, shoot P concentration and shoot dry mass were measured. Variation in colonisation within the core and among the cultivars was similar, ~12-68% of root length. However, 41% of the core lines had > 40% of root length colonised compared with 24% of the cultivars. There was a positive linear correlation between colonisation level and shoot P concentration (r2=0.36, P<0.0001), but not shoot dry mass. Relationships between colonisation level and specific root length or average root diameter were weak. We conclude that potential may exist to develop subterranean clover lines with higher or lower colonisation by AMF. However, the benefits of doing so must first be established under field conditions.

Key words
Pasture legumes, mycorrhizal fungi, phosphorus, core collection

Introduction

Subterranean clover (*Trifolium subterraneum* L.) is the most widespread annual pasture legume in the temperate pastures of southern Australia (Nichols *et al*., 2012). For these pastures, substantial savings in P-fertiliser use, and improvements in fertiliser P-use efficiency, may be possible if the plant-extractable P concentrations in soil could be reduced, but herbage yield maintained (Simpson *et al*., 2014). Plants with lower P requirements typically have roots with improved ability to forage P from soil (Lynch, 2011; Richardson *et al*., 2011). There are a number of root traits that assist P foraging, including the level of colonisation by arbuscular mycorrhizal fungi (AMF).

When subterranean clover is grown in pasteurised soil in a glasshouse, inoculation with AMF promotes colonisation of the roots and reduces the plant's critical P requirement (the amount of P needed for near-maximum growth) as a result of increased P uptake from low P soil (Abbott and Robson, 1977; Schweiger *et al*., 1995). In the pastures of southern Australia, colonisation of subterranean clover roots by AMF is ubiquitous and a diverse population of AMF is likely to be present (Abbott and Robson, 1982; Ryan and Kirkegaard, 2012; Simpson *et al*., 2011).

To examine the feasibility of modifying the level of AM fungal colonisation in subterranean clover, we examined the percentage of root length colonised by AMF for 42 cultivars and for a further 97 lines which constitute the "core collection". The core collection was developed from the 10 000 lines of subterranean clover which are available in genetic resource centres worldwide and it is estimated to represent almost 80% of the total diversity of the species (Nichols *et al*., 2013).

Method

The experiment examined the 139 lines of subterranean clover; 97 lines core lines (Nichols *et al*., 2013) and an additional 42 cultivar lines which were included as they would already possess many agronomically favourable characters. Overall, there were 35 lines of ssp. *brachycalycinum*, 97 lines of ssp. *subterraneum* and 7 lines of ssp. *yanninicum*. All seed originated from a single seed-bulking trial at Shenton Park Research Station (Floreat, Western Australia). The experiment was located in a glasshouse with a diurnal range of 5ºC to 24ºC. Sterilised pots (90 × 90 × 180 mm) were filled with 1 kg of an unpasteurised sandy-loam soil (8% clay, 82% sand, 10% silt). The soil had been collected from 0–40 cm depth in remnant forest, dried at 40ºC for one week and thoroughly mixed. It had low plant-extractable P (5.8 mg/kg, Colwell 1963) and a high P-buffering index (591) and was acidic (pH CaCl2 4.7). The soil was chosen as it was low in P, and a preliminary experiment had found subterranean clover grown in it was well colonised by AMF with no evidence of the major fungal root pathogens of subterranean clover. There were four replicate pots of each line. Seedlings were thinned to one per pot after one week. Phosphorus-free basal nutrients were applied. Pots were maintained at field capacity.

At harvest (week 6), root length (for calculation of specific root length) and average root diameter were measured after scanning fresh roots using the WinRHIZO 4.1 software package (Regent Instruments Inc., Quebec, Canada, 2000). The percentage of root length colonised by AMF was determined on a subsample of roots after staining in an ink/vinegar solution (Vierheilig *et al*., 1998). Shoots were dried at 60ºC for three days and weighed. Shoots were digested in a 3:1 HNO3:HClO4 solution and P measured by the yellow vanadomolybdate method.

Results

The percentage of root length colonised by AMF varied widely among the core lines from 15% to 55% (Fig. 1a). Colonisation in the cultivar lines covered a similar range, although one cultivar, Izmir, had 68% of root length colonised. The cultivars Izmir, Dalkeith (58%) and Bacchus Marsh (47%) were high colonisation outliers among the cultivar lines. Cultivars with very low colonisation, that is < 20% of root length colonised, were Dinninup, Trikkala, Rosedale, Larisa and Riverina.

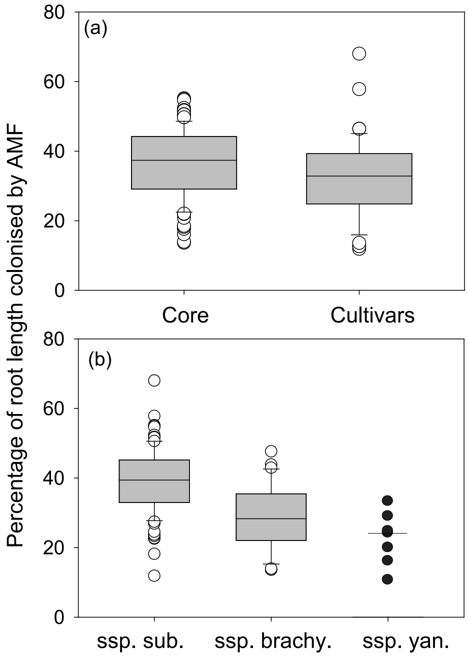

Figure 1. Box plots of the percentage of root length colonised by arbuscular mycorrhizal fungi (AMF) in T. subterraneum among: (a) core lines (n=97) and cultivar lines (n=42); and (b) ssp. *subterraneum* (sub.) (n=97), ssp. *brachycalycinum* (brachy.) (n=35) and ssp. *yanninicum* (yan.) (n=7). The boundaries of the box indicate the 25th and 75th percentiles, and the line within, the median. Whiskers indicate the 90th and 10th percentiles. Individual points are outliers. Subspecies yanninicum had insufficient data for a box plot and thus all data are graphed and the median shown.

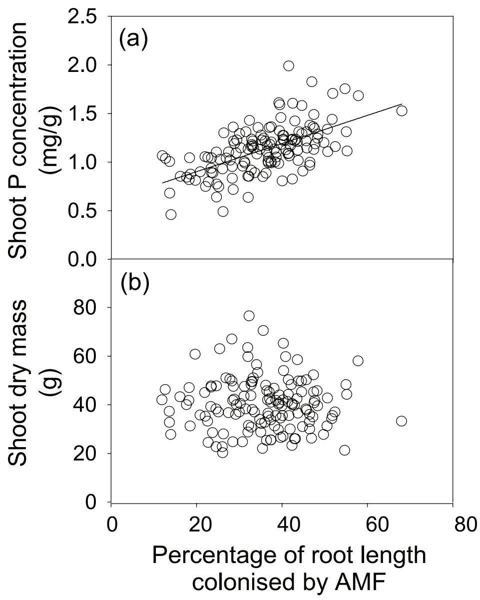

Figure 2. The correlation of (a) shoot P concentration and (b) shoot dry mass with the percentage of root length colonised by arbuscular mycorrhizal fungi (AMF) (n=139). For (a) r2=0.36, P<0.0001.

Overall, mean colonisation level was higher for the core lines than for the cultivar lines (core lines, 36.8% ± 1.0 s.e.; cultivar lines 32.8% ± 1.7 s.e; two-tailed t-test P=0.037). In addition, 41% of the core lines had >40% of root length colonised, while only 24% of the cultivar lines had >40% of root length colonised.

The percentage of root length colonised by AMF also varied among the three subspecies (Fig. 1b). Subspecies subterraneum was the most variable and included the 13 most highly colonised lines and the line with the lowest colonisation. Colonisation of ssp. *subterraneum* (39.2% ± 0.9 s.e.) was significantly higher than that of both ssp. *brachycalycinum* (28.5% ± 1.5 s.e.) and ssp. *yanninicum* (24.5% ± 3.0 s.e.) (two-tailed t-test P<0.0001). Colonisation did not differ between ssp. *brachycalycinum* and ssp. *yanninicum* (two-tailed t-test P>0.05). While no lines of ssp. *yanninicum* had >40% of root length colonised, 14% of ssp. *brachycalycinum* lines and 46% of ssp. *subterraneum* lines did so. Results for ssp. *yanninicum* may be influenced by the low number of lines representing this subspecies (n=7, all of which were cultivar lines).

Correlations between the percentage of root length colonised and shoot P concentration (Fig. 2a) and shoot dry mass (Fig. 2b) were investigated using the mean for each core line and cultivar line (i.e. n=139). There was a positive linear correlation between colonisation level and shoot P concentration, which ranged from 0.45–2.00 g/kg (Fig. 2a, r2=0.36, *P*<0.0001). There was also a positive linear correlation between the total length of colonised root and shoot P concentration (r2=0.35, *P*<0.0001; results not shown). There was no correlation between shoot dry mass and the percentage of root length colonised by AMF (Fig. 2b). Relationships between colonisation level and specific root length and average root diameter were significant, but as they accounted for relatively little of the variation in the data their practical significance is doubtful. Specific root length ranged from 85–175 m/g and had a positive correlation with the percentage of root length colonised by AMF (r2=0.14, *P*<0.0001). Average root diameter ranged from 0.295–0.424 mm and had negative linear correlation with the percentage of root length colonised by AMF (r2=0.13, P<0.0001).

Discussion

The differences in ranking among the subterranean clover subspecies were not anticipated and the reason is unknown; it could reflect differences in their ability to become colonised or be an indirect effect of differences in root morphology, root distribution or root growth rate. The comparison of the genotypes was made in a light textured, moderately acidic soil potentially more suited to the ssp. *subterraneum* and this may also have influenced the outcome. By comparison, ssp. *brachycalycinum* is noted for growth in neutral-alkaline soils (Nichols et al., 1996) and ssp. *yanninicum* prefers heavier, acidic soils (Nichols *et al*., 2013). However, it was evident that the ssp. *subterraneum* lines were not all highly colonised.

The slightly higher mean colonisation of the core lines (37% of root length) than the cultivar lines (33% of root length) is consistent with the recent meta-analysis of Lehmann et al. (2012) who found that ancestors, old cultivars and new cultivars of annual crop plants had a mean colonisation of 41%, 30% and 32%, respectively. However, other reports contradict this finding. An et al. (2010) found that the percentage of root length colonised among more than 200 lines of maize (*Zea mays* L.) was similar, perhaps even higher, for modern hybrids than older landraces. Leiser et al. (2015) found no effect of origin (landrace or researcher-bred) on colonisation levels in 187 sorghum (*Sorghum bicolor* L. Moench) lines from west and central Africa. Colonisation level was also found to have low heritability (Leiser et al., 2015).

Even if the heritability of colonisation level in subterranean clover is high, undertaking a breeding program targeting development of cultivars with higher levels of colonisation than current cultivars should be preceded by a robust study of the role of AMF in the growth and nutrition of subterranean clover under field conditions. There are a number of reasons for suggesting this approach. First, while we found a positive correlation between colonisation level and shoot P concentration, there was no correlation with shoot DM. Second, AMF have little impact on P uptake and growth of subterranean clover if plant-extractable-P (Colwell) is greater than ~20 mg/kg (Abbott and Robson, 1977; Schweiger et al., 1995). Third, several studies suggest little benefit for autumn-sown crops in southern Australia from high colonisation by AMF, even when under P limitation (Ryan and Kirkegaard, 2012). Similar studies are required for subterranean clover.

Conclusions

The percentage of root length colonised by AMF varied widely among core lines and cultivar lines of subterranean clover. However, it remains to be determined whether this variation has sufficient heritability to allow exploitation by breeders. The benefit to the growth and nutrition of subterranean clover of colonisation by AMF under field conditions in southern Australia also requires clarification. However, if higher, or lower, colonisation was shown to be desirable, the fact that cultivar lines had a similar range of colonisation to the core lines would mean that selection could occur from existing cultivars thereby encapsulating other agronomically-desirable characteristics such as disease resistance.

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