Phosphorus Use Efficiency and Partitioning Among Forage Legume Genotypes

B.M. Goff*[1], A.L. Fowler[2], E.K. Langlois[1], L.M. Lawrence[2], and S.R. Smith[1]

[1]*Department of Plant and Soil Sciences, University of Kentucky*

[2]*Department of Animal and Food Sciences, University of Kentucky*

**Corresponding Author: ben.goff@uky.edu*

ABSTRACT

Forage legumes require a higher level of soil fertility than many grasses species and the failure to provide these nutrients is one of the common reasons for the poor persistence of these species in forage systems. In addition, the cost of phosphorus (P) has risen in response to higher global demand and the diminishment of mined reserves which has reduced the amount of this fertilizer applied by producers. The objectives of this study were to examine the P use efficiency (PUE) of alfalfa (*Medicago sativa* L.), birdsfoot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pratense* L.), and white clover (*T. repens* L.) genotypes to evaluate the possibility of selecting for varieties with lower fertility requirements and to elicit possible mechanisms that led to the improved efficiency of this soil nutrient. Samples were collected from the University of Kentucky (UK) forage variety trial in May 2013 and June 2015 when all species were in the early bud stage of growth. Despite high soil P concentrations in several locations(> 400 kg P ha^{-1}), differences ($P < 0.001$) in PUE were observed between legume species and may represent differences in P utilization by the plant rather than the difference in P uptake. Red clover had the highest PUE followed by alfalfa and birdsfoot trefoil. White clover had the lowest PUE among the species evaluated. Higher PUEs across species were correlated with a greater partitioning of P towards the NDF and ADF fractions of the plant cell wall instead of storage forms (i.e. phytate). This indicates that there is potential to improve legume tolerance to lower soil fertility by selecting genotypes that place more emphasis on supplying nutrients to active growth. Alfalfa and white clover also exhibited genotypic differences in PUE ($P < 0.01$) that was related to stand maturities and levels of glyphosate tolerance.

INTRODUCTION

- Phosphorus (P) is frequently the soil nutrient that most limits the growth of forage legume species unlike grass species whose growth is typically N limited
- The taproot of most forage legumes offers a lower root surface area compared to grasses which allows the plant to extract P from a smaller volume of soil
- P availability is also regulated by various edaphic conditions including soil pH, texture, soil mineralogy, concentration of other nutrients, etc.
- Limited uptake P potential and low P availability may contribute to the poor persistence of forage legumes in forage-livestock systems of the southeastern U.S.
- In addition, the price of P fertilizers has risen due to concerns over the supply of remaining mineable P reserves and the over-application and general misuse of P fertilizers often leads to detrimental environmental consequences
- The objectives of this study were to examine the P use efficiency (PUE) of alfalfa (*Medicago sativa* L.), birdsfoot trefoil (Lotus corniculatus L.), red clover (*Trifolium pratense* L.), and white clover (*T. repens* L.) genotypes to evaluate the possibility of selecting for varieties with lower P requirements
- A sub-objective of this study was to examine the partitioning of shoot P to elicit possible mechanisms that led to the improved metabolic utilization of this soil nutrient

MATERIALS AND METHODS

- Samples were collected from the University of Kentucky (UK) Forage Variety Trial during May 2013 and June 2015 and correspond to the first and second harvest of each season
- One location was harvested in 2013 (Lexington, KY) and three locations were harvested in 2015 (Jackson, KY; Lexington, KY; and Princeton, KY) to obtain four different growing environments that provided a range in soil P concentrations (Table 1)
- Alfalfa and red clover genotypes were available in each environment, but white clover and birdsfoot trefoil genotypes were only available at the Lexington sites in 2013-2015 and 2015, respectively
- Dried samples were ground to pass through a 1-mm screen with a Udy mill and scanned (400-2500 nm) with a FOSS NIRSystems 6500 near-infrared spectrometer (NIRS)
- NIRS equations were developed for neutral detergent fiber (NDF), acid detergent fiber (ADF), and P concentrations and were selected based on low standard errors and high R^2 and 1-VR (> 0.90 & 0.70, respectively)
- A colorimetric method (Fiske and Subbarow, 1925) was used to quantify P concentrations in the samples and NDF/ADF fractions
  - Phytate (*myo*-inositol hexaphosphate) was quantified using the colorimetric assay described in Latta and Eskin (1980)
- An ANKOM fiber bag method was used to estimate NDF and ADF concentrations (Vogel et al., 1999)
- Data was analyzed using PROC GLIMMIX (SAS 9.3) as a CRD using a '/GROUP' to correct for unbalanced numbers between species and heterogeneous variances:
  - Environment and replication were treated as random effects
  - '/PDIFF' option in LSMEANS used to compare treatment means of quantitative factors
  - PROC REG used to compare the correlation between response variables

Table 1. Summary of soil test results for growing environments of the study.

Environment	Soil pH	Phosphorus	Potassium
		kg P ha^{-1}	*kg K ha^{-1}*
2013 Lexington, KY	6.0	417	212
2015 Lexington, KY	5.8	404	169
2015 Princeton, KY	6.3	23	138
2015 Jackson, KY	5.2	43	169

RESULTS AND DISCUSSION

Forage Yield, P Concentrations & P Use Efficiency:

- Alfalfa produced the highest forage yields followed by red clover, birdsfoot trefoil, and white clover when averaged across genotypes (Table 2)
- White clover forage had the highest P concentrations (4.56 g P kg DM^{-1}), while red clover forage provided the lowest amount of P (4.27 g P kg DM^{-1})
  - Alfalfa and birdsfoot trefoil forage contained P concentrations intermediate to the clover (Table 2)
- The low P concentrations and high yields resulted in red clover genotypes having, on average, higher PUE (242 g DM g P^{-1}) than the other legume species (Table 2)
- While white clover had the lowest PUE (218 g DM g P^{-1}), it does not necessarily imply that it is less efficient at extracting and utilizing soil nutrients than the other species as the lower PUE may be an artifact of the prostrate growth habit of the clover leading to lower forage yields
- The lower yields of white clover and birdsfoot trefoil allowed for less P removed during harvest (Table 2)
  - Alfalfa forage removed the greatest total P removed during harvest (12.54 kg P ha^{-1}) followed by red clover (8.70 kg P ha^{-1})

Table 2. Forage yield, P concentrations, P use efficiency (PUE), and total P removed during harvest of legume species averaged across genotypes (n). Letters refer to a difference in the means at $P < 0.10$.

Species	n	Yield	P Conc.	PUE	Tot. P
		kg DM ha^{-1}	*g P kg DM^{-1}*	*g DM g P^{-1}*	*kg P ha^{-1}*
Alfalfa	76	2,880***a***	4.39***b***	230***b***	12.54***a***
Birdsfoot Trefoil	10	660***c***	4.34***bc***	232***b***	2.92***c***
Red Clover	26	2,090***b***	4.27***c***	242***a***	8.70***b***
White Clover	26	320***d***	4.56***a***	218***c***	2.33***c***

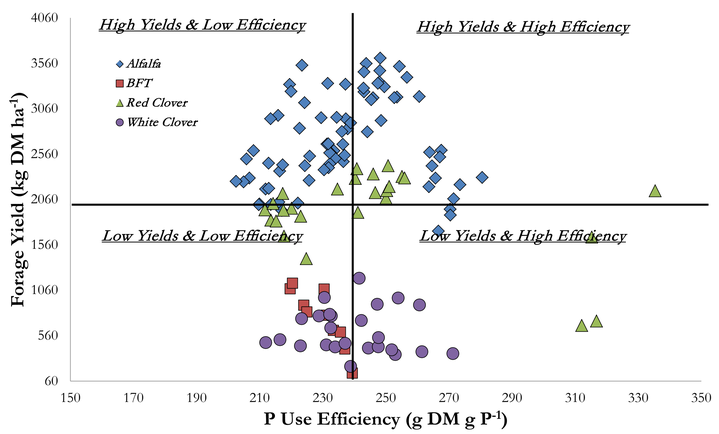

Figure 1. Forage yield and P use efficiency (PUE) alfalfa, birdsfoot trefoil (BFT), red clover, and white clover genotypes. Lines represent the mean yield (2020 kg DM ha^{-1}) and PUE (239 g DM g P^{-1}) averaged across all species.

- There was considerable intra-species variation ($P < 0.05$) in PUE and forage yields among the genotypes (Fig. 1) with exception of birdsfoot trefoil ($P = 0.11$) which may be due to fewer available genotypes
  - The total amount of harvested P varied ($P < 0.05$) among genotypes of all species (alfalfa: 7.19 – 17.74 kg P ha^{-1}, birdsfoot trefoil: 0.69 – 5.81 kg P ha^{-1}, red clover: 2.41 – 11.58 kg P ha^{-1}, white clover: 0.24 – 5.53 kg P ha^{-1})
- Improvements in PUE was highly correlated with increases in use efficiency of other macronutrients (data not shown)
  - This is suggestive that increases in PUE, to some extent, may be due to improvement in root architecture (i.e. greater root volume, higher root hair surface area, etc.)

P Partitioning Among Genotypes:

- The utilization of absorbed P varied less between species than total P and PUE (Table 2)
  - White clover genotypes had higher P in the neutral detergent soluble (NDS) fraction (79.7%) and greater phytate concentrations (5.5%)
  - Alfalfa genotypes had less P in the ADF fraction (1.0%) than the other species and had phytate concentrations (5.4%) similar to white clover
- Phytate was negatively correlated with PUE ($P < 0.001$; Fig. 2a) when compared across species
  - This was surprising given that phytate is a storage form of P but may indicate that some species may absorb P in levels higher than their requirement
  - High PUE genotypes may more utilized these reserves earlier in the season and/or stored less phytate in aboveground tissues
- Forages were only harvested once per growing season and the importance of phytate on PUE may occur over a longer time interval

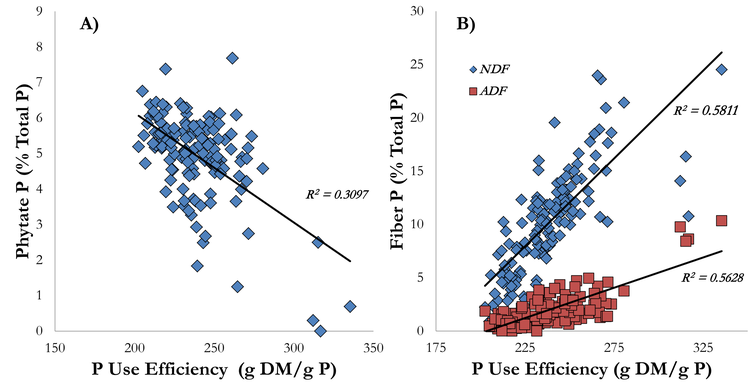

Figure 2. The correlations between phytate, neutral detergent fiber (NDF) P, acid detergent fiber (ADF) P and P use efficiency among forage legume genotypes

- Phosphorus use efficiency (PUE) was also correlated with higher P concentration in the cell wall fraction (i.e. NDF & ADF; Fig. 2b)
- This was initially believed to be an artifact of cell wall development with more efficient genotypes partitioning more photosynthate towards their cell wall
  - However while NDF & ADF concentrations were highly correlated with yield ($P < 0.001$; $R^2 > 0.40$), P concentrations in these fraction were weakly correlated ($R^2 < 0.15$) with forage yield or fiber concentration
- Several genotypes may have the ability to more effectively catabolize and remobilizedcell wall P than others
- Decreases in PUE associated with higher phytate concentrations may suggest that cell wall P may also be a function of *myo*-inositol metabolism
  - Several inositol di- & triphosphates regulate the production of many monosaccharides utilized in the production of hemicelluloses (Valluru and Van den Ende, 2011)
  - The balance of phytate & its metabolites may lead to altered cell wall composition which may effect the plant turgor required for cell expansion and development

SUMMARY

- Phosphorus use efficiency (PUE) varied between forage legume species but was somewhat confounded with species growth habit
  - ***Ex:*** White clover exhibited the lowest PUE (218 g DM g P^{-1}) among the species evaluated but removed the least amount of total P during harvest (2.44 kg P ha^{-1})
- Considerable intra-species in forage yield and PUE suggest that it may be possible to select for genotypes that exhibit more effective use of soil nutrients
- Synergistic improvements in use of other soil nutrients with PUE suggest that more efficient nutrient use may be a result of root architecture leading to great P ***uptake***
- However, the correlations between phytate and cell wall P (i.e. NDF/ADF P) with PUE also suggest that P ***utilization*** differs between genotypes
  - The precise nature of these associations are still being elucidated

Literature Cited

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