PHOSPHORUS USE EFFICIENCY FOR BIOMASS PRODUCTION OF LEGUME SPECIES

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ABSTRACT
The objective of the experiment was to identify traits associated with the production of plant biomass, and hence with P utilization efficiency, when legume species were fertilized.

Red clover (*Trifolium pratense)* and narrowleaf birdsfoot trefoil (*Lotus tenuis*) were compared in a greenhouse experiment using phosphorus deficient soils. Three plants grew in pots fertilized with 0, 2, 4, 8, 15, 20, 30 and 40 ppm in soil, in a randomized experimental design. Harvest was made at 60 days from the sowing date. Leaf areas, shoot and root biomass, and tissue phosphorus contents were measured. Results showed that changes in leaf area ratio in response to phosphorus fertilization influence nutrient use efficiency. *Lotus tenuis* showed a pronounced decreasing trend in these variables compared with *Trifolium pratense* ($P < 0.05$).

KEYWORDS
Phosphorus, phosphorus deficiency, fertilization, *Lotus tenuis L.*, *Trifolium pratense L.*

INTRODUCTION
During legume species establishment, since emergence up to active growth of stems and roots, phosphorus fertilization enhances their competitive ability (Tremmel and Bazzaz, 1995).
But increasing fertilizer costs demand that nutrient be used efficiently in agronomy practices. If some forage genotypes could produce adequate dry matter at low soil phosphorus levels, reduced fertilizer inputs may be required.

When different genotypes are being compared, the evaluation of biomass production and other variables associated, per unit of phosphorus absorbed, contemplate adequately the differences in nutrient internal requirements (Blair and Wilson, 1990). Shoot:root ratio, leaf area ratio (LAR) and leaf area (LA) are major determinants of total plant growth, and resource conversion in biomass. So, the hypothesis was that the increment in leaf area size relative to total biomass response is a key factor determining phosphorus use efficiency of legume species at increasing phosphorus supplies. The objective of the experiment was to identify traits associated with the production of plant biomass, and hence with P utilization efficiency, when legume species were fertilized.

METHODS
Red clover (*Trifolium pratense L.*) and narrowleaf birdsfoot trefoil (*Lotus tenuis L.*) which represent higher and lower nutrient external requirements respectively (Langer, 1990, Montes, 1988), were compared in a greenhouse experiment using phosphorus deficient soils from a location in the Flooding Pampa region (central east of Buenos Aires province), characterized by usual soil phosphorus deficiencies (Darwich, 1983).

Soils cores (total N: 0.26%, total C: 3.2%, pH: 6.7, extractable P: 2.7ppm), obtained from the upper horizon (10 cm) were dried, placed into polyethylene bags and mixed with a initial basal nutrient solution. Fertilization levels of 0, 2, 4, 8, 15, 20, 30 and 40 ppm in soil were obtained by adding phosphorus (P) as a solution of KH_2PO_4. Bags with two kg of soil were placed into pots without drainage. Legume seeds were Rhizobium inoculated, and sown in the pots, obtaining three homogeneous plants per pot. Daily watering maintained the soil at 80% of field capacity. Harvest was made at 60 days from the sowing date. Biomass was divided into shoots and roots, and leaf area per plant was measured using a leaf area meter. Then fresh material was dried for 48-72 hours at 70× C and weighed. Shoot and root P contents were determined in the dried samples by acid digestion (Haslemore and Rougham 1976).

Shoot biomass was expressed as dry matter per plant (g DM. plant^{-1}). The leaf area ratio was determined by the quotient between leaf area and total biomass produced (cm^2 g DM^{-1}), being an indicator of the proportion of photosynthetic active tissues in the total biomass produced (Lamber and Poorter, 1992). Biomass and leaf areas were expressed per unit of P absorbed to compare the nutrient utilization efficiency between both species.

A randomized experimental design was used, with three replicates. Data were analyzed by regression analysis with selection of polynomial degree -Stepwise method-. Analyses of variance and Tukey methods were used to determine statistical differences among mean values observed ($P < 0.05$).

RESULTS
At low levels of P supply (0, 2 and 4 ppm), *Lotus* showed significantly higher shoot biomass per unit of P absorbed than *Trifolium* (Table 1). The reduction was sharper for birdsfoot trefoil plants than for red clover plants, from its highest value at low P supplies to a very low value after the addition of 15-20 ppm.

LAR showed a different response pattern to increasing P supplies in *Lotus* and *Trifolium*. *Lotus* declining trend contrasted with an increasing one of *Trifolium,* when shoot:root ratios increased in response to P supply (Fig 1).

RESULTS AND DISCUSSION
Lotus tenuis showed a remarkable decline in phosphorus efficiency for shoot biomass production compared with *Trifolium pratense*. This trend was similar to that observed for leaf area per unit of P absorbed (cm^2. mg P^{-1} x 10^3):
LA -*Lotus*- = 0.360 - 0.281 dose + 0.0004 dose2 r2 0.92*
sd 0.046
LA -*Trifolium*- = 0.360 + 0.015 dose - 0.0003 $dose^2$ r^2 0.74*
sd 0.036

This confirmed that leaf area exerts a major influence in growth and in the internal utilization efficiency of the nutrients (Lambers and Poorter, 1992). This eminent decline in the leaf area per unit of P absorbed coincided with a great increase in the number of stems per unit of P absorbed
(N×. mg P^{-1} x 103)
Stems -*Lotus*- = 13.4 + 1.9 dose r^2 0.89* sd 2.1
Stems -*Trifolium*- = 1.7 - 0.0005 $dose^2$ r^2 0.62ns sd 0.3

Shoot growth costs are higher than those due to leaf generation (Mooney, 1972, Coyne et al., 1995), hence *Lotus* high dry matter gains per unit of P absorbed at reduced phosphorus levels, decreased probably as a consequence of higher respiratory costs. Efficiency reductions observed, associated to a scarce inversion in leaf area relative to the number of stems per unit of P absorbed, coincide with Poorter (1989), who had observed that LAR is the morphological and functional trait which explain most of the variation in the internal nutrient use.

Results showed that changes in leaf area ratio in response to phosphorus fertilization influence nutrient use efficiency. *Lotus tenuis* showed a pronounced decreasing trend in both variables compared with *Trifolium pratense.*

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Table 1
Plant shoot biomass per unit of phosphorus absorbed (g DM. mgP^{-1})

P supply	0	2	4	8	15	20	30	40
Clover	0.52^y	0.51^y	0.49^y	0.46^{xy}	0.42^{xy}	0.40^x	0.36^x	0.34^x
Lotus	0.82^x	0.79^x	0.75^x	0.49^{xy}	0.41^{xy}	0.17^y	0.13^y	0.15^y
SEM^z	0.001	0.003	0.005	0.003	0.001	0.004	0.006	0.009

^{z} Standard error of the mean (three observations per mean).
^{x,y} Values on the same column with different superscripts are different, P< 0.05.

Figure 1
Leaf area ratio -LAR - at different shoot : root ratios in response to phosphorus supply

y_2 *(Trifolium)* = -196.5 + 385.1 s : r - 125.1 s : r^2
r^2 0.95* sd 3.07

y_2 *(Lotus)* = -2497.0 + 2568.5 s : r - 620.9 s : r^2
r^2 0.97* sd 15.9