

Variation in growth and N₂ fixation within and among *Centrosema* spp. in response to phosphorus supply

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Abstract

Four inoculated ecotypes of each of *Centrosema acutifolium*, *C. brasilianum* and *C. macrocarpum* were grown in undisturbed soil (oxisol) cores in a glasshouse to study their response to 7 phosphorus levels (a total of 11, 22, 45, 90, 180, 360 and 720 mg/core P, surface applied as Ca(H₂PO₄)₂.H₂O).

Seven weeks after planting, dry matter production, leaf area, N yield, nodulation, nodule-P concentration and acetylene reduction activity increased strongly with P supply. Enhanced leaf area was due mainly to increases in individual leaf size, especially in the large-leaved *C. macrocarpum*. Shoot-N concentration of all species increased with P supply, which was attributed to increased nodule mass and N₂ fixation, but not to a greater proportion of leaf material.

The response to P supply in leaf number, leaf size and leaf-to-stem ratio as well as nodule number and weight differed among species. However, there was no significant (P > 0.05) species × P rate interactions for dry matter and N yield, shoot-N %, or nodule-P %, but strong intra-specific variation was found. All 3 *Centrosema* species contain important genetic variability in the growth and N₂ fixation responses to P supply during establishment. Therefore both parameters should be taken into account in selecting ecotypes.

Resumen

Cuatro ecotipos inoculados de cada una de las especies de *C. acutifolium*, *C. brasilianum* y *C. macrocarpum* fueron cultivados en invernadero en núcleos de suelo (Oxisol) sin disturbio con el fin de estudiar su respuesta a 7 niveles de fósforo (un total de 11, 22, 45, 90, 180, 360 y 720 mg/núcleo P aplicados a la superficie del suelo en forma de Ca(H₂PO₄)₂.H₂O).

La producción de materia seca, el área foliar, el rendimiento de nitrógeno, la nodulación, la concentración de P en los nódulos y la actividad de reducción de acetileno se incrementaron fuertemente a las 7 semanas después de la siembra con la aplicación de P. El aumento en el área foliar fue debido al incremento en el tamaño individual de la hoja, especialmente en la especie de hoja grande *C. macrocarpum*. La concentración de N en los rebrotes de todas las especies se incrementó con la aplicación de P; tal incremento es atribuido al aumento en la masa de nódulos y a la fijación de N₂, y no a la gran proporción de hojas.

Los cambios en el número y tamaño de hojas, relación hoja tallo, y en el número y peso de los nódulos como respuesta a P variaron dentro de las especies. Sin embargo, la interacción fósforo × especie no fue significativa (0 > 0.05) en cuanto a: rendimiento de materia seca y N, %N en los rebrotes y %P en los nódulos; pero se encontró una fuerte variación intra-específica. Dicha variación intra-específica mostró que las 3 especies de *Centrosema* contienen una variabilidad genética importante de los parámetros de crecimiento y fijación de N₂ en respuesta a la aplicación de P durante el establecimiento, pero en la selección de ecotipos tales parámetros debieran ser considerados.

Introduction

The genus *Centrosema* is comprised of about 35 recognised species of herbaceous tropical legumes. However, only *Centrosema pubescens* has attained economic importance as a forage plant and as a cover crop in plantation agriculture. Due to a lack of adaptation to acid soils with a high aluminium (Al) saturation, *C. pubescens* has not been successfully used on infertile oxisols and ultisols of tropical America. In recent years, scientists from the CIAT (International Center for Tropical Agriculture) Pasture Program have selected pasture plant germplasm for acid soils, including accessions of *C. acutifolium*, *C. brasilianum* and *C. macrocarpum* (Schultze-Kraft 1986; Schultze-Kraft *et al.* 1987; Schultze-Kraft and Belalcazar 1988).

Phosphorus deficiency is one of the most widespread constraints to growth of *Centrosema* on tropical soils (Salinas *et al.* 1990). *Centrosema pubescens* is considered to require more P for growth than *Stylosanthes guianensis*, *Pueraria phaseoloides* or *Desmodium ovalifolium* (Kerridge and Ratcliff 1982). Within *Centrosema*, *C. macrocarpum* had a lower internal and external P requirement for dry matter production than *C. pubescens* (CIAT 1982), while there were intra-specific differences for those attributes between accessions of *C. acutifolium*, *C. brasilianum* and *C. macrocarpum* (CIAT 1987; Salinas *et al.* 1990). Field evaluations showed a marked reduction in N₂ fixation due to P or

potassium deficiency, especially in *C. macrocarpum*, but only one ecotype of each of *C. acutifolium* and *C. macrocarpum* was compared (Cadisch *et al.* 1989; 1993).

The aim of the experiment reported here was to compare growth and N₂ fixation responses to P application within and between *Centrosema* species which have been selected for growth in acid soils. For this purpose, germplasm collected from different ecological zones in tropical America was compared.

Materials and methods

Four ecotypes of each of 3 *Centrosema* species originating from different regions of Colombia, Brazil and Venezuela were compared (Table 1). Three seedlings were planted in undisturbed soil cores of 0.1 m diameter × 0.25 m containing about 3 kg dry soil (CIAT 1988) in the glasshouse at Cali, Colombia. Soil cores were taken from a P-deficient oxisol of the Eastern Plains of Colombia. The soil had a sandy silt loam texture with a pH of 4.7, 896 mg/kg total N, 1.3 mg/kg P (Bray II) and an Al saturation of 80%. Legumes were inoculated with a mixture of effective *Bradyrhizobium* strains, CIAT No. 49, 1670, 1780, 3101, 3196 and 3694 (CIAT 1989). Water content of cores was maintained at 80% of field capacity.

Seven P treatments (11, 22, 45, 90, 180, 360 and 720 mg/core P) as Ca(H₂PO₄)₂·H₂O were applied to the soil surface. All pots received a

Table 1. Origin of *Centrosema* ecotypes.

CIAT accession number	Origin	Soil-P status	Ecological zone
<i>C. acutifolium</i>			
5277	Colombia: Vichada	— ¹	Urban
5568	Brazil: Goias	medium ²	Cerrado
15086	Colombia: Vichada	high	Urban
15445	Venezuela: Amazonas	medium	Gallery forest
<i>C. brasilianum</i>			
5178	Venezuela: Bolivar	medium	Savanna
5234	Brazil: Bahia	low	Cerrado
5712	Venezuela: Anzoategui	medium	Savanna
15286	Brazil: Mato Grosso	—	—
<i>C. macrocarpum</i>			
5065	Colombia: Meta	—	—
5452	Colombia: Casanare	—	Dry forest
5592	Brazil: Goias	—	—
5713	Venezuela: Anzoategui	low	Savanna

¹ Indicates no information available.

² Units (mM P) are: low, < 0.16; medium, 0.16–0.48; high, > 0.48.

basal fertilisation (in mg/core) of: 108 K, 93 S (K_2SO_4), 54 Mg (18 MgO and 36 $MgSO_4 \cdot 7H_2O$), 270-0 Ca ($CaCO_3$ to complement Ca in the low P supply), 4.5 Zn ($ZnSO_4 \cdot 7H_2O$), 0.9 Cu ($CuSO_4 \cdot 5H_2O$), 0.9 B ($Na_2B_4O_7 \cdot 10H_2O$) and 0.5 Mo ($Na_2MoO_4 \cdot 2H_2O$). Both $CaCO_3$ and MgO were incorporated into the surface soil layer 2 weeks before sowing, and micronutrients were applied in a solution 1 day before sowing. The doses of all other macronutrients were split: one third of the total amount was applied as solution at the beginning of the experiment and the remainder was given in equal amounts weekly on 6 consecutive occasions to achieve more constant differences in soil P availability with time in this high P-fixing soil (Le Mare 1982).

Plants were harvested 7 weeks after planting. Nodulated roots were incubated for 30 minutes in 585 ml serum bottles containing 0.1 atm. acetylene, and gas samples were analysed for ethylene by gas chromatography. Shoot samples and tissue bulked from 4 replications of roots and red nodules (pink to red colour) were dried at 65°C and analysed for total N (Kjeldahl) and P as described by Salinas and Garcia (1985). The cores were arranged in a split-plot design (4 replications) with the fertilisation treatment as the main plot and the ecotypes as randomised subplots. The data were analysed using a nested model (ecotypes within species) with the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA). The following equation was fitted

$$y = a - b \exp(-cx) \quad (1)$$

where y is the yield of tops (mg/core), x is the P applied (mg/core P), a is the estimated maximum value for y , b is the range between the estimated maximum and estimated value of y when no fertiliser was applied, and c is the curvature coefficient. At any point on the curve the response (efficiency) to increased P supply is given by the slope of the curve (Barrow 1975). Differentiating (1) gives:

$$dy/dx = b \times c \exp(-cx) \quad (2)$$

Results

Yield and leaf development

Yield of shoot dry matter increased strongly with increasing P application in all ecotypes (Figure 1). Differences in the average maximum yield between the 3 *Centrosema* species were small, but there were large differences in yield among

ecotypes. The strongest intraspecific variation in maximum dry matter yield was observed among ecotypes of *C. acutifolium*. The species \times P rate interaction for dry matter yield was not significant ($P > 0.05$). However, there was considerable variation in the curvature (coefficient c), the yield response (coefficient b) (Figure 1) and the calculated P efficiency values (Figure 6). Despite an often lower shoot-to-nodulated-root ratio observed in *C. macrocarpum*, the ability to take up P at low P levels was not significantly different between species (not presented).

The yield response to increasing P supply was associated with an increase in total leaf area per plant of 247% (average of all ecotypes). The leaf area responses in *C. brasilianum* were related to leaf number, whereas in *C. macrocarpum* it was leaf size (Figure 2a). Specific leaf weight was highest in *C. acutifolium* (data not shown) and decreased from 3.58 to 3.20 mg/cm² with increasing P rate. However no species \times P rate interactions occurred. The leaf-to-stem ratio decreased as P fertilisation increased from 11 to 180 mg/core P (Figure 2b). The decrease was greatest in *C. acutifolium* which had the highest leaf-to-stem ratio at low P supply.

Nitrogen accumulation, nodulation and acetylene reduction

Shoot-N concentration increased significantly ($P < 0.05$) with P rate (data not shown), and with enhanced shoot-P concentration (Figure 3). Large increases in N yield with a small increase in shoot-P concentration were observed (Figure 4), but the species \times P rate interaction for N yield (log transformed data) was not significant. Near-maximum N yield was often reached at higher shoot-P concentrations in ecotypes of *C. brasilianum* than in the other species. Phosphorus fertilisation increased average N yield by 299% (average of all ecotypes) compared with an increase in shoot dry matter production of 239%.

Phosphorus fertilisation increased total nodule weight by an average of 331% and nodule number by 197% (Figure 5), and the species \times P rate interactions were significant. With *C. acutifolium*, nodule mass reached a maximum at 180 mg/core P but further increases in nodule mass up to the highest P rate were observed with *C. brasilianum*. Nodule number of *C. brasilianum* was least at the lowest P rate and

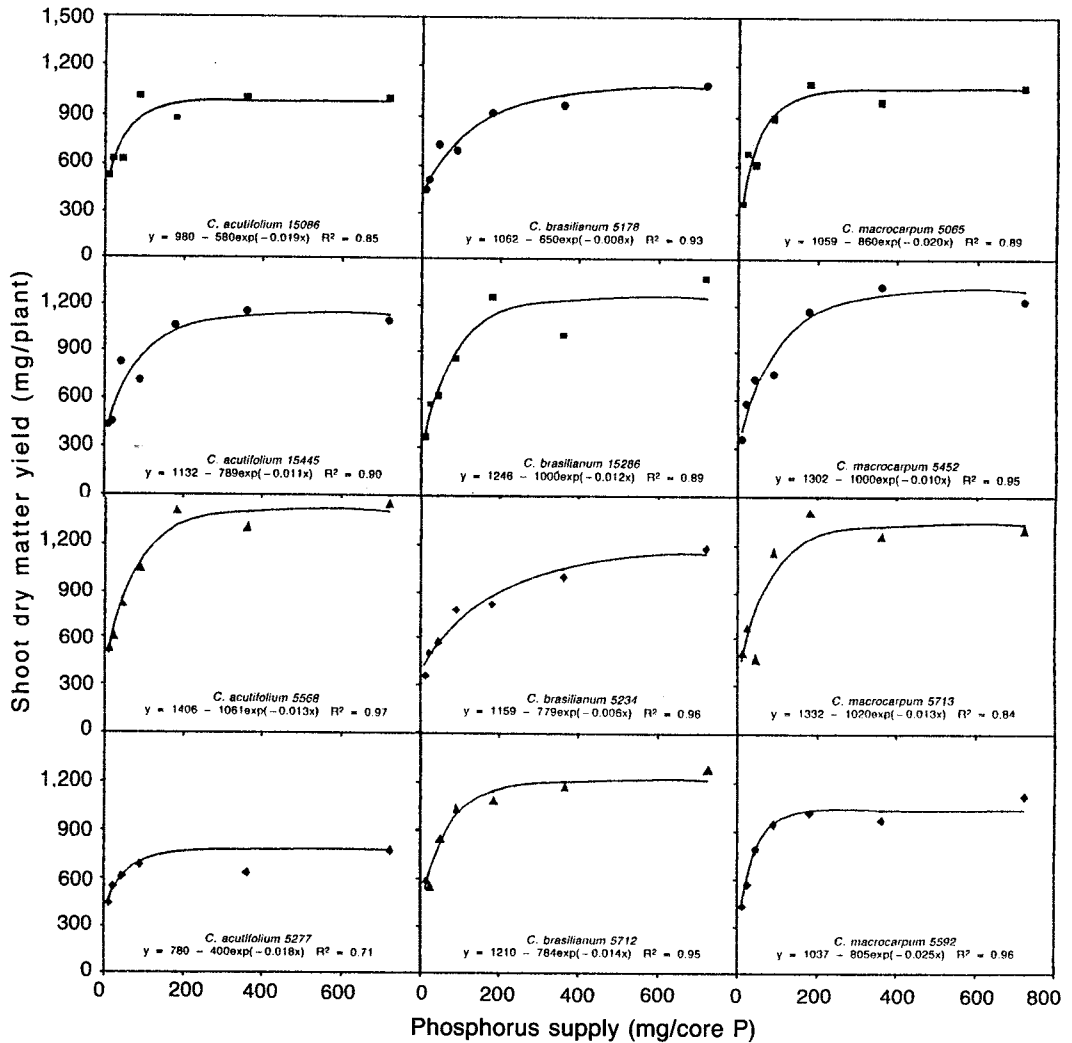


Figure 1. Shoot dry matter yield as affected by P supply of four ecotypes (CIAT No.) of each of 3 *Centrosema* species. Curves were fitted to $y = a - b \exp(-cx)$.

gradually increased to similar values to those of the other species at 360 mg/core P.

Phosphorus concentration of nodule tissue was strongly correlated with P concentration of shoots ($y = 0.14 + 0.57x$, $R^2 = 0.95$, average of all ecotypes). At low P supply the P concentration of the nodule (0.21%) was considerably higher than that in the shoot (0.14%); ecotype 15286 had a very high nodule-P concentration (0.30%). Rates of acetylene reduction per plant increased from 3.0 to 8.0 $\mu\text{M}/\text{h}/\text{plant}$ C_2H_4 (average of all ecotypes) with increasing P supply. However, acetylene reduction on a nodule mass basis did

not increase significantly with P addition (data not shown).

Discussion

Species and ecotype variation in the growth response to P

The large P response in yield confirms that one of the main growth limiting factors in this soil is P deficiency. Similar responses to P application have been observed during establishment in

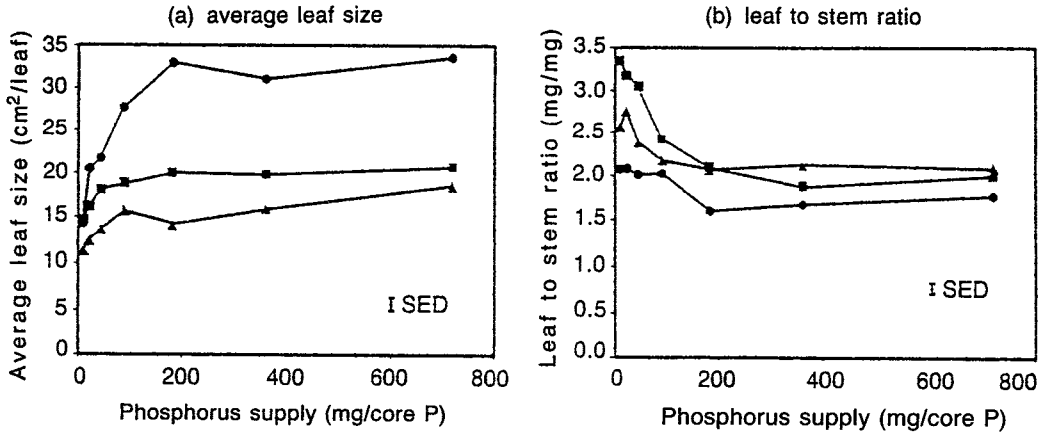


Figure 2. Effect of phosphorus supply on average leaf size (a) and leaf to stem ratio (b) of *C. acutifolium* (■), *C. brasilianum* (▲) and *C. macrocarpum* (●), averaged across 4 ecotypes for each species. SED = Standard Error of Mean Difference.

a previous field experiment, which also confirmed the higher yield potential of ecotype 5452 compared to 5277 under conditions of adequate P supply (Cadisch *et al.* 1993). The ability of the tested *Centrosema* species to respond strongly to P fertilisation suggests that they are well adapted for growth in this acid soil with its high Al saturation, since there was no lime applied at the higher P rates. While large differences in the yield response to P between ecotypes were observed, general differences between the 3 species investigated were smaller or non-existent during this early stage of growth. Thus observed differences in previous field experiments between ecotypes of *C. acutifolium* and *C. macrocarpum* (Cadisch *et al.* 1989; 1993) cannot be attributed to general differences between species, but are rather due to intra-specific variation. The P responses obtained here do not seem to be related to the soil P status at the site of origin; *Centrosema* ecotypes 5234 and 5713 were collected from low P soils but were able to respond well, but differently, to applied P. Similarly, Schultze-Kraft (1986) observed that accessions of *C. macrocarpum* grew very vigorously when fertilised regardless of the soil fertility at their site of origin. Observed differences between ecotypes were mainly in relation to P efficiency (slope of the response curve) and maximum yield rather than to differences in growth at the lowest P level; similar observations were recorded in *Stylosanthes* (Jones 1974). Thus selection based on growth under low soil P conditions may be difficult. The small response to added P shown by *C. acutifolium* 5277, an

ecotype recently released as a cultivar (cv. Vichada), could be attributed to adaptation to low soil fertility as suggested by Caradus and Snaydon (1986). Good persistence (Thomas and Grof 1986) and less yield depression of established 5277 plants under a combined P and K deficiency compared to *C. macrocarpum* 5065 (Cadisch *et al.* 1989) suggest that it may be better adapted to low soil P or K. For improved grass-legume pastures, on less P-deficient soils or with P maintenance fertilisation, ecotypes with a high P efficiency (i.e. a steep response curve) such as ecotype 5568, 5712 or 5592, might be preferential because, in mixture with P responsive grasses, P supply favours grass dominance (Coates *et al.* 1990). Ecotypes with such a behaviour can be found in the 3 species tested. While our results obtained under controlled conditions agree well with observed responses in the field during establishment, it remains to be determined if this holds in established pastures.

When plants are deprived of P, decreases in leaf area are generally observed before the rate of photosynthesis per unit is affected (Hart and Greer 1988; Fredeen *et al.* 1989). Our results confirm the strong effect of P on total leaf area. Fredeen *et al.* (1989) also observed reduced leaf size at low P supply and concluded that, for soybean, this may be due to a lack of phosphate for the expansion of epidermal leaf cells. This may explain why the effect of P deficiency on leaf expansion of *C. macrocarpum*, which had the biggest leaves, was the strongest. We found also a positive relationship between P efficiency (slope

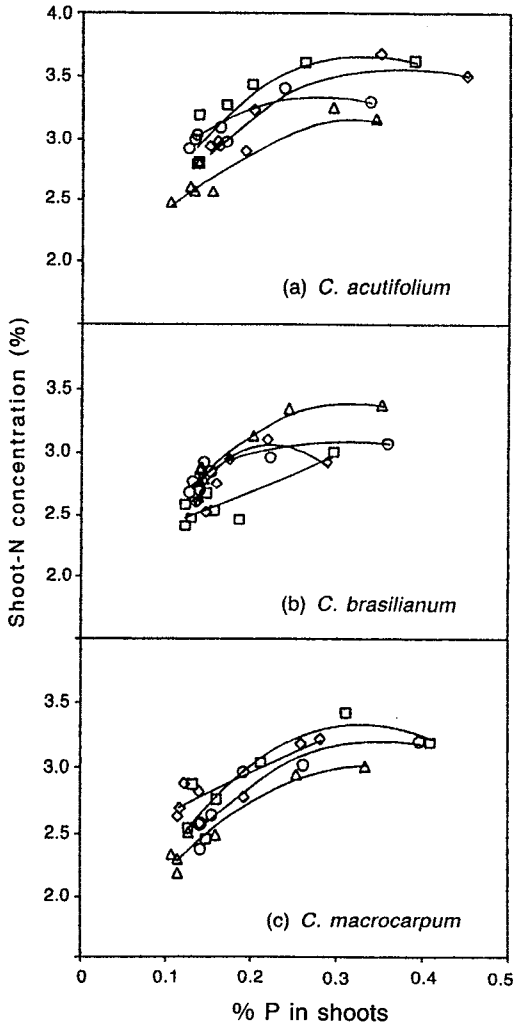


Figure 3. Nitrogen concentration in shoot tissue as related to P concentration in shoot tissue of ecotypes (symbols as for Figure 1) of *C. acutifolium* (a), *C. brasilianum* (b) and *C. macrocarpum* (c). Each point represents the mean of 4 ecotypes and one P treatment. Curves were fitted to $y = a + bx + cx^2$.

of the growth curve) at the lowest P level and leaf size (Figure 6) which further emphasises the effect of P on leaf expansion. However, while there appeared to be a general relationship between leaf size and P efficiency, within species this held only for *C. acutifolium*. Stress-adapted plants, such as native legumes found in the savannas, often have small leaves, as has *C. acutifolium* 5277, and it might be worth testing if this is an adaptation to P-deficient conditions.

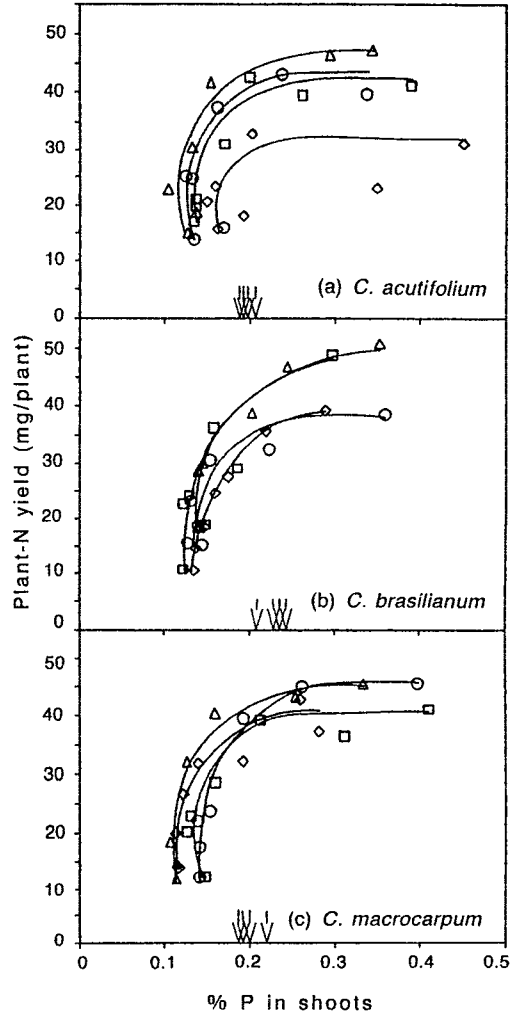


Figure 4. Total N accumulation (shoots and roots) in relation to P concentration in shoot tissue of ecotypes (symbols as for Figure 1) of *C. acutifolium* (a), *C. brasilianum* (b) and *C. macrocarpum* (c). Each point represents the mean of 4 ecotypes and one P treatment. Curves were hand-fitted. Arrows indicate shoot-P concentration for 90% of maximum yield.

Species and ecotype variation in N₂ fixation

Increases in the N concentration in shoots were not associated with a higher proportion of leaves, since the leaf-to-stem ratio decreased with enhanced P supply. However, increases in N concentration were related to nodule mass and reached a maximum value when nodule mass was near its maximum (Figure 7). Apart from nodule mass being more strongly affected by P deficiency than was shoot dry matter, nodule mass also

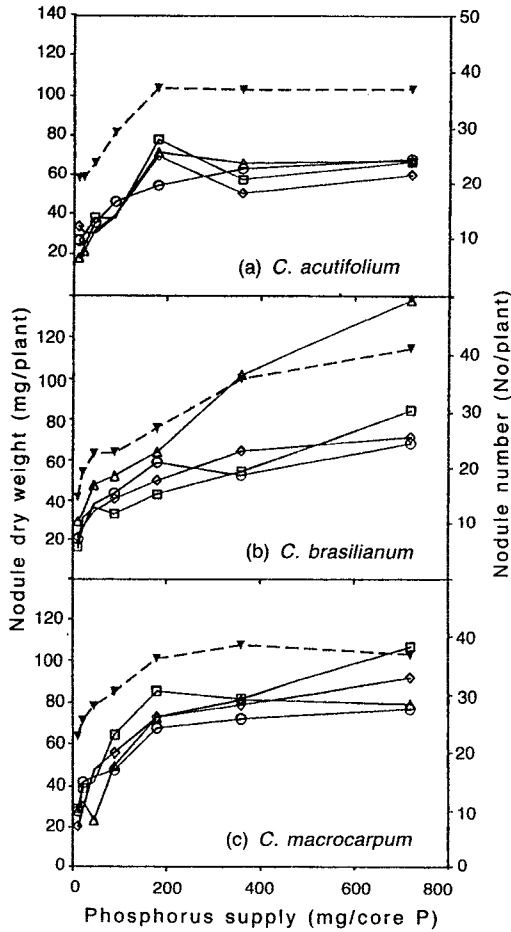


Figure 5. Effect of P supply on total nodule weight (—) of ecotypes (symbols as for Figure 1) of *C. acutifolium* (a), *C. brasilianum* (b) and *C. macrocarpum* (c) and on average nodule number (-----) of species. $SED_{ecotype (spp.)}$ for nodule weight = 9.2 mg; $SED_{species}$ for nodule number = 2.5.

responded less quickly (lower curvature coefficient) to improved P supply. Thus nodule mass was near its maximum at a higher rate of P application than was shoot yield. This suggests that, apart from the effect on growth, there was a specific effect of P on nodule growth and hence N_2 fixation of *Centrosema* species improving shoot N concentration. The results confirm previous field observations where P supply enhanced not only growth and N concentration but also the proportion of N derived from fixation (Cadisch *et al.* 1993). The importance of good modulation is shown by ecotype 5712 where

the very abundant nodule mass led to the highest N concentration and N yield in shoots. The observation that nodules are a strong sink for P at low P supply is explicable by a very active metabolism due to the high energy requirement for N_2 fixation. However, a high allocation of P into nodules at low P supply, as observed in *C. brasilianum* 15286, appeared not to be associated with a high ability to fix N_2 .

Variation in N concentration and yield occurred between and within *Centrosema* species. The observed species \times P interaction for nodulation was associated with the often higher P concentration in shoots needed for near-maximum N yield in *C. brasilianum*, but did not lead to a species \times P rate interaction. Screening only for growth parameters does not always lead to the selection of good N_2 fixation ability, which is especially important in mixed swards where grasses strongly compete for available soil mineral N. *Centrosema acutifolium* 5568 has promising P response yield attributes; however, it did not appear in this experiment to be a very good N_2 fixer, as shown by low N concentration (2.6%) in shoots associated with poor nodulation (18 mg/plant) at the lowest P supply. In contrast, *C. acutifolium* 5277 had a high N concentration (3.0%) associated with good nodulation (33 mg/plant) leading to a very favourable ratio of shoot to nodule mass. Its high ability to fix N_2 , even under P stress, was confirmed under field conditions (Cadisch *et al.* 1993). A large intra-specific variation in N_2 fixation can often be attributed to host-strain interactions (Graham 1982; Jones 1974) but this factor should not have interfered here as a mixture of effective strains for *Centrosema* spp. was applied. However, 3 *C. macrocarpum* accessions, and *C. acutifolium* 5568 showed much more marked responses to rhizobial inoculation in the field than ecotype 5277 or 5234, because the latter 2 accessions nodulated more effectively with native strains (Sylvester-Bradley *et al.* 1990). Possibly *Bradyrhizobium* strains that are more effective at low P supply need to be selected for the Rhizobium-specific *Centrosema* ecotypes. Within *C. brasilianum*, ecotype 5712 appears to be the ideal choice, and should be preferred over 15286; it showed similar yield performance to the latter, but had higher N_2 fixation at all P levels. Thus, when performing intra-specific selection, the ability to fix N_2 , as well as dry matter yield attributes, should be taken into account.

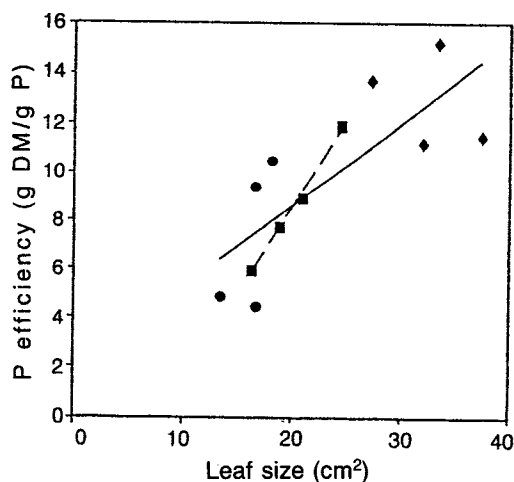


Figure 6. Relationship between P efficiency (= slope of dry matter response curve in Figure 1) and average leaf size of *C. acutifolium* (■), *C. brasilianum* (▲) and *C. macrocarpum* (●). Average (—) $y = 1.76 + 0.34x$, $R^2 = 0.61$; *C. acutifolium* (-----) $y = -6.15 + 0.73x$, $R^2 = 0.99$.

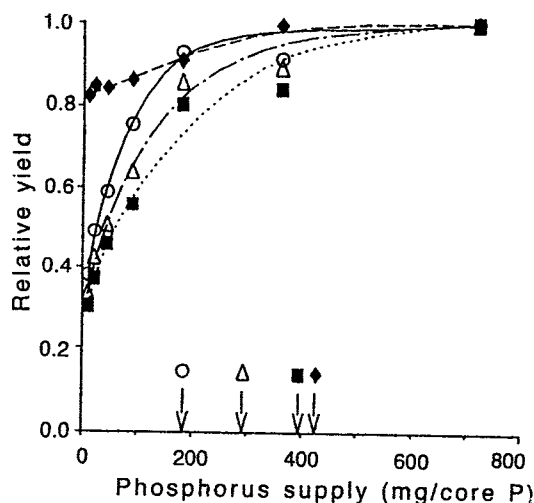


Figure 7. Relative responses to P supply for overall average shoot dry matter (O, $y = 1 - 0.69 \exp(-0.0116x)$, $R^2 = 0.98$), total N accumulation (Δ , $y = 1 - 0.71 \exp(-0.0078x)$, $R^2 = 0.98$), nodule mass (■, $y = 1 - 0.73 \exp(-0.0059x)$, $R^2 = 0.98$) and shoot N concentration (◆, $y = 0.816 + 0.00068x - 5.9 \times 10^{-7} x^2$, $R^2 = 0.98$). Arrows indicate P requirement for 90% of maximum yields except for shoot N concentration where arrow is for maximum ($y = 1$) value.

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References

- BARROW, N.J. (1975) The response to phosphate of two annual pasture species. I. Effect of the soil's ability to adsorb phosphate on comparative phosphate requirement. *Australian Journal of Agricultural Research*, **26**, 137-143.
- CADISCH, G., SYLVESTER-BRADLEY, R. and NÖSBERGER, J. (1989) ¹⁵N based estimates of nitrogen fixation by eight tropical forage legumes at two levels of PK-supply. *Field Crops Research*, **22**, 181-194.
- CADISCH, G., SYLVESTER-BRADLEY, R., BOLLER, B.C. and NÖSBERGER, J. (1993) Effects of phosphorus and potassium on N₂ fixation (¹⁵N dilution) of field grown *Centrosema acutifolium* and *C. macrocarpum*. *Field Crops Research*, **31**, 329-340.
- CARADUS, J.R. and SNAYDON, R.W. (1986) Response to phosphorus of populations of white clover. 1. Field studies. *New Zealand Journal of Agricultural Research*, **29**, 155-162.
- CIAT (1982) Fertilidad de Suelos y Nutrición de Plantas. *Informe Anual 1981. Tropical Pastures Program, CIAT, Cali, Colombia*. pp. 171-194.
- CIAT (1987) Soil/Plant Nutrition. *Annual Report 1986. Tropical Pastures Program, CIAT, Cali, Colombia*. pp. 198-218.
- CIAT (1988) Legume-rhizobium symbiosis: Methods manual for evaluation, selection, and agronomic management. *Soil Microbiology Section of the Tropical Pastures Program and Soil Microbiology Section of the Bean Program (comps), CIAT, Cali, Colombia*.
- CIAT (1989) Relative effectiveness of tropical forage legume-rhizobium combinations. Catalogue of results of greenhouse and field trials. *Working document No. 49, CIAT, Cali, Colombia*.
- COATES, D.B., KERRIDGE, P.C., MILLER, C.P. and WINTER, W.H. (1990) The effect of phosphorus on the composition, yield and quality of legume-based pasture and their relation to animal production. *Tropical Grasslands*, **24**, 209-220.
- FREDEEN, A.L., RAO, M.I. and TERRY, N. (1989) Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. *Plant Physiology*, **89**, 225-230.
- GRAHAM, P.H. (1982) Plant factors affecting symbiotic nitrogen fixation in legumes. In: Graham, P.H. and Harris, S.C. (eds) *Biological Nitrogen Fixation Technology for Tropical Agriculture*. pp. 27-37. (CIAT: Cali, Colombia).
- HART, A.L. and GREER, D.H. (1988) Photosynthesis and carbon export in white clover plants grown at various levels of phosphorus supply. *Physiologia Plantarum*, **73**, 46-51.
- JONES, R.K. (1974) A study of the phosphorus responses of a wide range of accessions from the genus *Stylosanthes*. *Australian Journal of Agricultural Research*, **25**, 847-862.
- KERRIDGE, P.C. and RATCLIFF, D. (1982) Comparative growth of four tropical pasture legumes and guinea grass with different phosphorus sources. *Tropical Grasslands*, **16**, 33-40.
- LE MARE, P.H. (1982) Sorption of isotopically exchangeable and non-exchangeable phosphate by some soils of Colombia and Brazil, and comparisons with soils of southern Nigeria. *Journal of Soil Science*, **33**, 691-707.
- SALINAS, J.G. and GARCIA, R. (1985) *Metodos Quimicos para el Analisis de Suelos Acidos y Plantas Forrajeras*. (CIAT: Cali, Colombia).
- SALINAS, J.G., KERRIDGE, P.C. and SCHUNKE, R.M. (1990) Mineral nutrition of *Centrosema*. In: Schultze-Kraft, R. and Clements, R.J. (eds) *Centrosema: Biology, Agronomy and Utilisation*. pp. 119-150. (CIAT: Cali, Colombia).

- SCHULTZE-KRAFT, R. (1986) Natural distribution and germplasm collection of the tropical pasture legume *Centrosema macrocarpum* Benth. *Angewandte Botanik*, **60**, 407-419.
- SCHULTZE-KRAFT, R., BENAVIDES, G. and ARIAS, A. (1987) Recolección de germoplasma y evaluación preliminar de *Centrosema acutifolium*. *Pasturas Tropicales*, **9**, 13-20.
- SCHULTZE-KRAFT, R. and BELALCAZAR, J. (1988) Germplasm collection and preliminary evaluation of the pasture legume *Centrosema brasilianum* (L.) Benth. *Tropical Agriculture (Trinidad)*, **65**, 137-144.
- SYLVESTER-BRADLEY, R., SOUTO, S.M. and DATE, R.A. (1990) Rhizosphere biology and nitrogen fixation of *Centrosema*. In: Schultze-Kraft, R. and Clements, R.J. (eds) *Centrosema: Biology, Agronomy and Utilisation*. pp. 151-174. (CIAT: Cali, Colombia).
- THOMAS, D. and GROFF, B. (1986) Some pasture species for the tropical savannas of South America. II. Species of *Centrosema*, *Desmodium*, and *Zornia*. *Herbage Abstracts*, **56**, 512-525.

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