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Phosphorus Nutrition and Mycorrhizal Growth Response of *Lotus corniculatus* and of *Lotus glaber*

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ABSTRACT

The effect of increasing P nutrition and mycorrhizal growth response of *Lotus corniculatus* and of *Lotus glaber* were studied on a soil of low P availability where the two species usually colonize. In terms of P utilization, *L. corniculatus* performed better than *L. glaber*. It produced larger yield of shoot per unit of P in shoot tissue. The critical P concentration measured as %P in shoot required to achieve 90% of maximum shoot yield was 0.22 in *L. corniculatus* and 0.28 in *L. glaber*. The roots of the two species were heavily infected by vesicular-arbuscular micorrhizal (VAM), and both, mycorrhizal and non-mycorrhizal plants responded strongly to added P in soil. The two species showed similar values for mycorrhizal dependency, 1.74 for *L. corniculatus* and 1.84 for *L. glaber*. The confidence intervals for these estimates values overlapped suggesting that the two species have no advantage or disadvantage each other to produce shoot yield for mycorrhizal relative to non-mycorrhizal plants. The fraction of roots colonized by VAM fungi differed between species at low level of added P but was similar at high added P. The specific root length (cm/g) of mycorrhizal plants was greatest in *L. corniculatus* when P was insufficient to achieve maximum growth. The lowest critical P concentration and the highest specific root

length at low levels of P nutrition of *L. corniculatus* suggest that the ability of *L. glaber* to spread and to grow in a wide range of soil is not related with its ability to grow better than *L.*

corniculatus in soils of low P availability. Previous works suggested that the tolerance to grow in flooding areas may be one of the properties involved.

INTRODUCTION

The genus *Lotus* is being increasingly employed for pastures around the world because of its productivity on wide range of soils. The importance of the *Lotus* species in Chile, Uruguay, Canada and USA has been reported by Seany and Henson (1970). *L. corniculatus* is widely grown as a forage legume in USA (Grant and Marten, 1985). In New Zealand, *L. corniculatus* and *L. pedunculatus* have been reported as important for pastures in soils of low P availability (Davies, 1991). In Argentina, two species of *Lotus* are important for pastures, broadleaf birdsfoot trefoil (*L. corniculatus*) and narrowleaf birdsfoot trefoil (*L. glaber*). Both legumes constitute an important forage resource for beef and dairy cattle farms in the Pampas of Buenos Aires.

The narrowleaf trefoil, *Lotus glaber* Mill. (syn. *L. tenuis* Waldst. Et Kit.), is a naturalized legume spread in a range of soils of Argentina's Pampas including, flooding areas, and heavy and nutrient deficient soils. The other important *Lotus* specie in Argentina is *L. corniculatus* L. It colonizes same areas that *L. glaber* but showed a lower expansion (Mazzanti et al., 1988; Vignolio et al., 1995). At least there are two reasons have determined that *L. corniculatus* is being lower used for pasture than *L. glaber*. One is the high capacity of *L. glaber* to spread naturally (Mazzanti et al., 1988; Vignolio et al., 1994; 1995), and the other is the hypothesis that the greater spread of *L. glaber* than *L. corniculatus* in a wide range of soils may be related with the ability to grow better at low levels of P availability in soil. Unfortunately, there have been few

experiments in which the comparison between the two species have been studied in an attempt to explain the mayor presence of *L. glaber* on a wide range of soils. The few evidences reported suggested that *L. corniculatus* had both, lowest recuperation and seed germination after flooding (Vignolio et al., 1994; 1995); and other evidence is that *L. corniculatus* grew better than *L. glaber* at low P availability in soil (Mendoza and Gigli, 1995). In addition, Davies (1991) found that *L. corniculatus* had a better growth than other pasture legumes (not *L. glaber* but *L. pedunculatus* included) at low P availability, suggesting that one reason would be an efficient association with a vesicular-arbuscular micorrhizal (VAM).

Vesicular-arbuscular mycorrhizal fungi can increase plant growth by increasing the uptake of P by plant at low P availability (Tinker, 1978). Most of the soils in the Pampas are low in P availability for pasture growth (Darwich, 1983), and the roots of *L. corniculatus* and *L. glaber* have showed an important VAM infection in a range of soil (Picon, 1984; Mendoza and Gigli, 1995). In addition, *L. glaber* showed a dependency for mycorrhizal infection to absorb P from the soil (Mendoza and Pagani, 1995), but unfortunately there is no previous report comparing the two *Lotus* species in order to know their presence in soil of low P availability.

Next to Nitrogen, P ranks as the nutrient most widely deficient in many part of the Argentinean Pampas. There is, therefore, a need for legumes capable of growing well under conditions of low soil P availability. In addition, due to there are few experiments in which *L. corniculatus* and *L. glaber* are studied, it would be interesting to compare their relative performances influenced by P nutrition. The aim of this work was to study and to compare the performance of *L. corniculatus* and *L. glaber* to grow at a range of P availability in soil. Then, if the performance between species differ, to relate the results with aspects of the VAM-plant association.. The aspects studied were: i) the ability to grow at a range of P availability insufficient to achieve maximum growth, ii) the mycorrhizal dependence to absorb P from a

typical soil where the two species usually colonize, and iii) to identify differences between species in properties that may be involved in P nutrition.

MATERIALS AND METHODS

Soil Used

The soil was a Typic Natraquoll from Chascomús (south Buenos Aires Province, Argentina) over which grew many communities of both *Lotus corniculatus* and *Lotus glaber*. Previous experiments with this soil have showed strong responses to added P by *L. glaber* and *L. corniculatus* as well as a heavy vesicular-arbuscular infection in roots by *Glomus* sp. (Mendoza and Gigli, 1995). Some properties of the soil are: pH in 0.01 M calcium chloride, 5.2; organic matter, 4.0 %; total nitrogen, 0.25 %; exchangeable calcium, magnesium, potassium and sodium, 12.4, 2.5, 0.6 and 1.0 mequiv. per 100 g respectively; extracted P (Bray 1) 3.5 mg kg⁻¹ of soil, and relative sorption capacity, 37 mg P kg⁻¹ of soil (Mendoza and Barrow, 1987).

Relative Growth Response of Phosphorus for Mycorrhizal and Non-mycorrhizal Plants

The top soil was collected and either fumigated with methyl bromide or left non sterile. Undrained pots of 1.6 L were filled with 760 g of soil (dry weight) fertilized with several amounts of H₂KPO₃ in solution in order to describe adequately the P response curve (0 - 200 mg P kg⁻¹ of soil). Fifteen mL of a filtrate through a 37 μ m sieve from a dilution (1:10) of non-fumigated soil was added to the pots of fumigated soils. A basal doses of nutrients except P (Ozanne et al., 1969) were mixed with the soil to ensure that plant growth was limited only by P. Sterilized sand was placed on top of the soil of each pot to form a band of 1.5 cm thick, in order to decrease soil water evaporation. Surface sterilized (alcohol 95 % plus H₂O₂ in 100 vol) of *L.*

corniculatus and *L. glaber* were germinated in a humid environment and six pre-germinated seedlings per pot were planted and grown in a greenhouse for 35 days. The pots were maintained near field capacity (33 % w/w) by watering daily and bringing them to a constant weight thrice a week. Tops and roots were harvested separately and weighed.

The Mitscherlich equation was used to fit the response curve of both mycorrhizal (M) and non-mycorrhizal (NM) plants to P applications (Abbott and Robson, 1991; Mendoza and Pagani, 1995):

$$Y = A - B \exp (-C X) \quad (1)$$

where Y is plant yield per pot at any level of added P (X), A is the maximum yield when P does not limit growth, A-B is the yield without P, and C is the curvature constant. Coefficient C is commonly related to the adsorption capacity of the soil, and an effective mycorrhizal infection is expected to change the value of C. When P does not limit growth, mycorrhizal and non-mycorrhizal plants do not differ in growth approaching the same maximum yield (A). The value of B is similar for mycorrhizal and non-mycorrhizal plants in soils that are low in native P (Abbott and Robson, 1991), in soils with higher status of P availability, B may be smaller for mycorrhizal than for non-mycorrhizal plants (Stribley et al., 1980). Where A and B approach to a same value for mycorrhizal and for non-mycorrhizal plants, Eq.(1), can be written,

$$Y = A - B \exp ((-C_M X_1) + (-C_{NM} X_2)) \quad (2)$$

where X_1 and X_2 are the levels of added P for mycorrhizal (M) and non-mycorrhizal plants (NM) respectively. In terms of efficiency of P applications for plant growth, the ratio between the

slopes of the response curves C_{NM}/C_M represents a measure of the relative effectiveness (RE) between non-mycorrhizal and mycorrhizal plants. Eq. (2), can be written as follow,

$$Y = A - B \exp (-C_M (X_1 + RE X_2)) \quad (3)$$

where RE is the relative effectiveness, and $C_{NM} = RE.C_M$. The RE is the reciprocal of the substitution rate (K) that represents the amount of source 2 (X_2) required to give the same effect on yield as a given amount of source 1 (X_1). In terms of mycorrhizal association, the value of K represents the dependence of the host on mycorrhizas in a specific soil (Abbott and Robson, 1987). Equation (3) was used to calculate the values of the coefficients to estimate the measures of C_M , C_{NM} , RE and K.

Oven dried (70 °C for 48 hs) shoot tissue was digested in nitric:perchloric acid (3:1), and P was determined by the molybdovanado phosphoric acid method (Jackson, 1958).

Critical Phosphorus Concentration

The critical P concentration in shoot to give 90% of maximum shoot growth was calculated from a rescaled version of the Mitscherlich equation used previously by Barrow and Mendoza (1990),

$$y = a (1 - \exp (-x^d c)) \quad (4)$$

where y is the dry weight of shoots (g), x is the concentration of P in shoots (%), and a, d and c are coefficients. The flexibility of this equation can be found in a previous paper (Barrow and

Mendoza, 1990). This equation is able to describe adequately sigmoidal response forms, and was used to calculate the critical P concentration in shoot for the *Lotus* species.

Mycorrhizal Infection

Measurements of mycorrhizal infection were done in fresh roots cleared in 10 % KOH for 30 minutes at 90 °C, and stained in lactic-glycerol blue. Root length was determined by the line intercept method and expressed as percentage of root length colonized.

The procedure used to distinguish the morphological features of the infection and identify the spores were as described by Abbott and Robson (1978; 1979), Abbott (1982), Morton (1988), and Hall and Abbott (1988). On this basis, the most prevailing VAM fungus was a *Glomus* sp. It resembled *Glomus fasciculatum* (Thaxter) Gerdemann and Trappe, (Hall, personal communication) . No further attempts were done to differentiate VAM fungi present.

The effect of supplying P on VAM infection was described by a non linear peak equation used in a previous experiment (Mendoza and Pagani, 1995):

$$R = Z / 1 + ((X^n - D)/E)^2 \quad (5)$$

where the coefficient Z represents the maximum value of root length infected (R) that is reached, when the dose of added P (X) raised to the power n is equal to D. Increasing additions of P, the value of R decreases at a rate controlled by coefficient E.

Statistical Analysis

The comparison between the two species of *Lotus* were analyzed by fitting curves. The statistical difference between curves were tested by a significant variation ($P < 0.05$) of the residual sum of squares of observed values. When the compared equations differed in number of coefficients, the net effect was that one equation of fewer coefficients was preferred if the change of the residual sum of squares was not significant ($P < 0.05$). The simplex method of Nelder and Mead (1965) was used to locate the values of the coefficients that gave the smallest residual sum of squares. Plant yield was log transformed for this analysis.

The confidence interval of the estimates values of the relative effectiveness (RE) or mycorrhizal dependency (K) was calculated using a program with varied the value of this parameter in Eq. (3), until the residual sum of squares of deviations was significant ($P < 0.05$) increased. More details of this statistical comparison can be found in a previous paper (Barrow and Mendoza, 1990).

RESULTS

Mycorrhizal and non-mycorrhizal plants responded strongly to addition of P in the soil (Fig. 1). Non-mycorrhizal plants required more added P to obtain the same yield as mycorrhizal plants, except at the lowest and the highest levels of added P. At these two levels, mycorrhizal and non-mycorrhizal plants approached to a same values of shoots growth. Hence, differences in shape between response curves fitted by Eq. (2) were characterized by differences in the value of coefficients C_M and C_{MN} (Table 1).

The benefit in shoot yield from the mycorrhizal association as assessed by the effectiveness of P growth response (RE) for non-mycorrhizal related to mycorrhizal plants (C_{NM}/C_M) was about 0.574 and 0.543 for *L. corniculatus* and *L. glaber* respectively (Table 1). These values represent a mycorrhizal dependency ($K = 1/RE$) of 1.742 and 1.842, suggesting that non-mycorrhizal plants required near 1.74 or 1.84 times more added P to obtain the same yield of mycorrhizal plants (Table 1). If the levels of added P for non-mycorrhizal plants (Fig. 1), are divided by 0.574 and 0.543, and thus expressed in added P for mycorrhizal plants equivalents, the points fall on a common fitting curve (Fig. 2).

The confidence interval of the estimates values of the relative effectiveness (RE) or mycorrhizal dependency (K) overlapped, suggesting no difference ($P < 0.05$) in RE and K between species (Table 1). This indicates that the two *Lotus* species have no difference in the ability to obtain the added P from the soil by mycorrhizal plants relative to non-mycorrhizal plants.

The relationship between the P concentration in shoots and the dry weight of shoots was the same for mycorrhizal and non-mycorrhizal plants (Fig. 3). This suggests that for each species separately, the difference of growth in the shape of the response curve reflects differences in the uptake of P rather than its utilization within the plant. This relationship was sigmoidal (Fig. 3), and fitted adequately by the rescaled version of the Mitscherlich equation discussed by Barrow and Mendoza (1990). When P in shoot was insufficient to reach maximum growth, the slope of the relationship differed between species (Fig. 3). At low and intermediate levels of P in shoot, *L. corniculatus* performed better than *L. glaber*. That is, for the same value of P concentration in shoot, both the yield and the slope of the relationship were greatest for *L. corniculatus* (Fig. 3). The critical P concentration calculated by Eq. (4) as the concentration of P in shoot required to

achieve the 90% of maximum shoot yield, was 0.22 and 0.28 for *L. corniculatus* and *L. glaber* respectively (Fig. 4).

Phosphorus nutrition affected differently the fractions of roots colonized by *Glomus* sp., (Fig. 4). For *L. corniculatus* the percentage of roots length infected initially increased at the first four low levels of added P, and thereafter was reduced with further additions. For *L. glaber* the maximum value of root length infected was when P was not applied, thereafter the fraction infected decreased with increasing P additions until reaching similar values of infection for the two species.

Root characteristics of mycorrhizal plants were affected by the level of added P and of mycorrhizal infection (Fig. 5). The specific root length increased with increasing the percentage of root length infected. At low levels of infection where P was sufficient for maximum growth, *L. corniculatus* and *L. glaber* had a similar value of specific root length. When the percentage of root length infected increased, and P nutrition was insufficient for maximum growth, *L. corniculatus* had a greatest value of specific root length (Fig. 5). This means, that at low levels of added P, the roots of *L. corniculatus* were longer and thinner than the roots of *L. glaber*. This may explain the ability of *L. corniculatus* to grow better than *L. glaber* at low P availability in soils.

DISCUSSION

This work shows that *L. corniculatus* and *L. glaber* differed in the ability to grow at a range of P availability in a soil where they usually are present at field. *L. corniculatus* produced larger yield of shoots per unit of absorbed P at low, medium and high P availability in soil. When P availability was insufficient to achieve maximum growth, the rate of increase of shoot yield per

unit of P in shoot was highest for *L. corniculatus* approaching maximum yield quicker. In terms of P utilization, *L. corniculatus* performed better than *L. glaber*. This result is consistent with previous work (Mendoza and Gigli, 1995), and suggest that the ability of *L. glaber* over *L. corniculatus* reported to spread and to grow in a wide range of soils (Mazzanti et al., 1988; Vignolio et al., 1994; 1995), may not be related with the ability to grow at low levels of P availability in soil. In the current experiment *L. corniculatus* had a lower critical P concentration value than *L. glaber*. It was reported that *L. corniculatus* had a very low critical P concentration comparing with other legumes (Davies, 1991). This ability may have contribute to its superior performance over *L. glaber* at low P availability in soil. In addition, Davies (1991) suggested that colonization of the roots system by an appropriate mycorrhizal fungi may be one of the reason of the better performance over other pasture legumes at low levels of P nutrition.

The roots of *L. corniculatus* and *L. glaber* were heavily infected by VAM fungi. The most prevailing VAM fungus identified was *Glomus fasciculatum*. The shape of the mycorrhizal response curve relative to the non-mycorrhizal curve showed a micorrhizal dependency (K) to absorb the added P from the soil. The values for micorrhizal dependency obtained in the current experiment were close to that obtained by Mendoza and Pagani (1995) for *L. glaber* (K=1.5). The dependence of the host on mycorrhizas have been calculated to range from 1.1 (no mycorrhiza benefit) to 5.8 (a large benefit to micorrhiza), in forage plants (Abbott and Robson, 1987). The values of mycorrhizal dependency to absorb P in the current soil by *Lotus* would be moderate (K=1.84).

The confidence intervals for the estimates values of RE and K overlapped (Table 1), suggesting that RE or K did not differ significantly between the *Lotus* species. This may suggest that the association between *Glomus fasciculatum* and *Lotus* is not specific for each specie. Previous results suggested that *L. corniculatus* and *L. glaber* did not have each other any

advantage or disadvantage to obtain added P that was incubated with the soil relative to a freshly added P (Mendoza and Gigli, 1995). The present result showed that the two *Lotus* species did not differ in the ability to obtain freshly added P from the soil by mycorrhizal plants relative to non-mycorrhizal plants. This contrasts the idea that mycorrhizal infection may be one of the reasons of the better performance of *L. corniculatus* over other pasture legumes at low levels of P nutrition (Davies, 1991).

For each of the two *Lotus* species, the relationship between concentration of P in shoots and shoot yield was the same for mycorrhizal and non-mycorrhizal plants suggesting that differences in growth reflect differences in uptake P rather than its utilization within the plant. Mendoza and Pagani (1995) have observed similar relationships.

The fraction of roots colonized by VAM fungi was affected differently by low levels of phosphorus nutrition. For *L. corniculatus* the percentage of root length infected initially increased at the first four low levels of added P, and thereafter was reduced with further additions. For *L. glaber* the maximum value of root length infected was when P was not applied, thereafter the fraction infected decreased with increasing P additions until reaching similar values of infection for the two species. This result is consistent with previous experiments in other legumes (Pairunan et al., 1980; Same et al., 1983; Abbott et al., 1984), but in *Lotus* as well (Mendoza and Gigli, 1995; Mendoza and Pagani, 1995). Adding P to the soil has been reported either to increase (Abbott and Robson, 1977; Bolan et al., 1984; Mendoza and Gigli, 1995), to decrease (Jensen and Jakobsen, 1980; Braunberger et al., 1991) or to have no effect (Anderson et al., 1987; De Miranda et al., 1989) on the level of VAM infection in a range of plants. This is the third report where the effect of different directions was observed (Mendoza and Gigli, 1995; Mendoza and Pagani, 1995). That is, an increase of the infection at low levels of added P and then a consistent decrease with further additions. The ability of VAM fungi to increase plant

growth depends on the P status of the soil. The soil used in the current experiment was low in P status and mycorrhizal infection did not increase growth when P was not applied. In fact, mycorrhizal infection increased P uptake and growth at intermediate rates of added P. When P availability is markedly deficient for plant growth, small additions of P to the soil have been shown either to increase (Abbott et al., 1984; Bolan et al., 1984; Mendoza and Gigli, 1995; Mendoza and Pagani, 1995) or to decrease (Thomson et al., 1986; Andreoli et al., 1997) the percentage of root length infected by VAM fungi. For the current specific relationship among soil-VAM fungi-host-period of growth, the most suitable explanation of the effect of P supply to increase infection at the first four low P levels in *L. corniculatus*, is that the growth of the VAM fungi is limited at low P. Then, little increase of P supply promotes an increase of the infection at a rate greatest relative to the rate of roots growth in soil (Pairunan et al., 1980; Same et al., 1983, Mendoza and Gigli, 1995), consequently, the percentage of root length infected increased. Further increase in P supply changes the relationships between the rate at which infection increases and the rate at which the roots growth, and the direction of the effect may change. It is argued, the direction of the effect will be controlling at least by four factors: the level of P availability in soil, the rate of increase of the fungus within the root, the rate of increase of the root within the soil, and the period of growth used for testing the effect. Depending the interactions among those factors, the percentage of root length infected may increase, decrease or no change with increasing added P at low levels.

Other important advantage to explain the better performance of *L. corniculatus* over *L. glaber* at low levels of P nutrition, is the differences between the species in the specific root length. At low levels of added P, the roots of *L. corniculatus* were longer and thinner than the roots of *L. glaber*. This would permit an advantage to absorb P from the soil and may explain the

ability of *L. corniculatus* over *L. glaber* and other legumes reported in previous experiments (Davies, 1991; Mendoza and Gigli, 1995).

The magnitude of the differences in P nutrition between the two *Lotus* species would have important implications in terms of absorbing P from added P fertilizers on P deficient soils. In terms of P utilization, there are enough evidences to conclude that *L. corniculatus* performed better than *L. glaber*. Thus, the ability of *L. glaber* over *L. corniculatus* reported to spread and to grow in a wide range of soils would not be related with the ability to grow at low P availability as most of the soil of the Pampas in soil have. In addition, the natural VAM-plant association would be not related either.

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Table 1. Comparison of values and confidence limits ($P < 0.05$) for the relative effectiveness (RE) and mycorrhizal dependence (K) of added P of non-mycorrhizal relative to mycorrhizal plants growth for *L. corniculatus* and *L. glaber* as measured by Eq.(3).

Plant specie	RE	Confidence limits		Range	K	Confidence limits		Range
		minimum	maximum			minimum	maximum	
<i>L. corniculatus</i> ^a	0.574	0.469	0.701	0.232	1.742	1.426	2.132	0.706
<i>L. glaber</i> ^b	0.542	0.435	0.679	0.244	1.845	1.472	2.298	0.826

a. $\log Y = 0.361 - 2.829 \exp(-0.0471(X_1 + 0.574X_2))$, $R^2 = 0.964$.

b. $\log Y = 0.071 - 2.829 \exp(-0.0513(X_1 + 0.542X_2))$, $R^2 = 0.956$.

Legends of the figures

Figure 1. Curves fitted to describe the response to added P for mycorrhizal and non-mycorrhizal plants by *L. corniculatus* and *L. glaber*. The estimates of C_M , C_{NM} , RE or K are given in Table 1.

L. corniculatus, $\log Y = 0.361 - 2.829 \exp(-0.0471(X_1 + 0.574X_2))$, $R^2 = 0.964$.

L. glaber, $\log Y = 0.071 - 2.829 \exp(-0.0513(X_1 + 0.542X_2))$, $R^2 = 0.956$.

Figure 2. Curve fitted to describe the response to added P for mycorrhizal and non-mycorrhizal plants by *L. corniculatus* and *L. glaber* with the levels of added P for non-mycorrhizal plants divided by RE and thus expressed as the added P for mycorrhizal plants equivalents.

Figure 3. Relationship between the concentration of P in shoots and the dry weight of shoots for mycorrhizal (closed symbols) and non-mycorrhizal (open symbols) plants by *L. corniculatus* and *L. glaber*. Eq. (4) was used to fit the curves drawn.

Figure 4. Effect of the added P on the percentage of roots length infected by *L. corniculatus* and *L. glaber*. Eq. (5) was used to fit the curves drawn.

Figure 5. Description of the relationship between the specific root length as a function of the percentage of roots length infected by *L. corniculatus* and *L. glaber*. The form of the function

used was a simple potential equation ($y = ax^b$); where y is the specific root length, x the percentage of roots length infected, and a and b are coefficients).

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