Rev. Fac. Agron. (LUZ). 2005, 22: 133-145

Comparative effects of two forage species on rhizosphere pH and solubilization of phosphate rocks of different reactivities

M. J. Pérez[1] y T. Jot Smyth[2]

[1]Recursos Agroecológicos, INIA-CENIAP, Apdo. Postal 4669, Maracay 2101-A, Aragua, Venezuela.
[2]Department of Soil Science, North Carolina State University, Raleigh, NC 27695-7619.

Abstract

Low soil pH and low concentrations of Ca and P in solution are the main soil factors controlling dissolution of phosphate rocks (PRs). Plant roots may affect PR solubility by extrusion of H^+ or OH^- in the rhizosphere. The objective of this study was to compare the effects of a grass (*Brachiaria decumbens*) and a legume (*Stylosanthes guianensis*) forage species on rhizosphere acidification and solubilization of three phosphate rocks (PRs) of different reactivities in a P- and Ca-deficient Ultisol. The experiment was conducted in a growth chamber at 30/26 °C day/night temperature, 12/12 hours light/dark period and 30% relative humidity. It consisted on a factorial combination of two forage species, two soil pH levels (original pH 4.9 and limed to pH 5.8 with $MgCO_3$) and five P treatments, arranged in a split plot design with three replications. Phosphorus treatments consisted on 50 mg P kg^{-1} of soluble P in neutral ammonium citrate, from Monte Fresco PR (MFPR), Riecito PR (RPR), North Carolina PR (NCPR), monocalcium phosphate reactive grade [$Ca(H_2PO_4)_2$] as a P soluble source (TSP), and a control without P. Pots were designed to isolate rhizosphere and non-rhizosphere soil. Rhizosphere soil pH decreased under Stylosanthes but increased under Brachiaria. Rhizosphere acidification and larger PR dissolution by Stylosanthes was associated with a larger root surface area, greater Ca uptake, and dependence on N_2 fixation by the legume, which may result in an excess of cathions in plant and induce H^+ extrusion from roots into the rhizosphere. Solubilization of RRs was influenced by the interaction of forage species and mineral composition of the PR. The MFPR has less apatite and higher content of $CaCO_3$ than RPR and NCPR, which corresponded to the high soil pH values (pH >7.0) and exchangeable Ca, and low dry matter production with MFPR treatments. Acidification of the rhizosphere by Stylosanthes is not

Received 11 27, 2003 ● Accepted 6 30, 2004
[1]Authors' e-mail: [1]mjperez@inia.gov.ve; [2]jot_smyth@ncsu.edu

sufficient to promote dissolution of the MFPR. Riecito PR performs better than NCPR as soil acidity increases
Key words: rhizosphere, phosphate rocks, forage species available P.

Introduction

It has been reported that some species of plants as well as legumes and oleaginous can significantly acidify the rhizosphere, doing them more efficient in the use of P of phosphoric rocks (1, 2, 3, 4, 5). These species have the ability to active certain physiologic H^+ and/or organic acids towards the rhizosphere to keep the internal balance of charges and the cellular neutrality of the plant.

On the other hand, in acid soils with deficient P, it is common to observe a huge proliferation of rootlets in some plants. In some plants with deficient P, the root/aerial area's relation is in most cases higher than in plants without deficient P. The radical system becomes the main storage organ of photosynthesized and of P transported from the aerial part of the plant, and is characterized by a high number of respiration, therefore by a high demand of oxygen (6). The increased in a superficial root area of plants with deficient P can be considered as an strategy to explore a higher volume of soil and solubilize P of the soil (6). Hinsinger and Gilkes (4) reported that some legumes as white lupine (*Lupinus albus* L.) have a huge capacity of dissolving phosphoric rocks, and they suggest that this might be related to the highest biomass of roots or to the highest activity of rootlets of this specie in relation to others species.

The aim of this study was to compare the effect of two forage species (*Brachiaría decumbens* vs. *Stylosanthes guianensis*) on the acidification of the rhizosphere, and the dissolution of three RFs of different chemical and mineralogical composition.

Materials and methods

Design and handle of the experiment

The experiment was done on a growth chamber, under the following environmental conditions: 30/26ºC day/night, 12/12 hours light/darkness and 30% relative humidity. Special pots were used, designed to isolate soil from the rhizosphere and the non-rhizosphere (figure 1). The pot is constituted by three divisions: the superior and inferior divisions are constituted by two tubes of polyvinyl chloride (PVC) of 17 cm of internal diameter and 7 cm height. These divisions were connected to a central division, which consisted on two perforated sheets of PVC where were inserted 20 tubes or columns of PVC of 15 mm of internal diameter. On these columns plastic tubes of 11 mm of internal diameter were inserted,

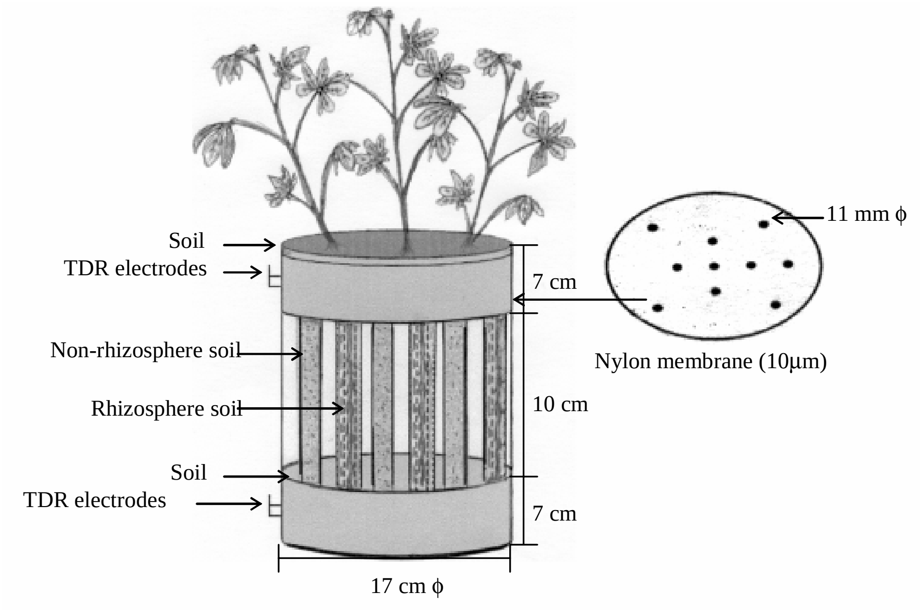

Figure 1. Diagram of the used pot used in the experiment to isolate soil of the rhizosphere from soil of the non-rhizosphere

plenty with the same soil-treatment that occupies the superior and inferior division of the pot.

On the superior area of the central division a membrane with nylon of 10 mm was put (Spectra/ Mesh, Spectrum Labs) and hollows in ten of the twenty tubes were perforated, which allowed that roots could go from the superior division to the inferior division trough half the columns of the soil. It was considered soil of the rhizosphere the soil in tubes, where roots extended to the inferior area of the pot, and soil of the non-rhizosphere the one located in tubes where the entrance of roots was blocked. The experiment consisted on a factorial arrangement of lime-coated P, and forage species treatments, located on a divided split plot design with three replications. The forage species *Brachiaría decumbens* (gramineae) and *Stylosanthes guianensis* (legume) represented the main smallholdings, and the secondary smallholdings were represented by a combination of five treatments of P and two pH levels of the soil (pH 4.9 and 5.8). P treatments consisted on applying 50 mg kg^{-1} of soluble P, coming from the phosphate rocks Monte Fresco (RFMF), Riecito (RFR) and North Carolina (RFCN) and reactive monocalcium phosphate [$Ca(H_2PO_4)_2$] as a reference of a source of soluble P (SFT), plus a control without P. The RF quantities required to supply 50 mg kg^{-1} of soluble P were calculated on the quantity base of soluble P in neutral ammonium citrate of each RF. The two pH levels of the soil were: the original pH of the soil 4.9, and lime-coated until pH 5.8, using 0.15 cmol kg^{-1} of Mg as $MgCO_3$. A sandy soil classified as Arenic Paleudults deficient in P and Ca (4 mg kg^{-1} of P-Olsen and 0.14 cmol kg^{-1} of interchangeable Ca) was used. Soil moisture was kept on field capacity during all the research, using a reflectometer of electrical impulse known as TDR. The Stylosanthes seeds were inoculated applying a specific inoculo suspension for Stylosanthes (15g of inoculo for Stylosanthes/150 mL of distilled water). A basic fertilization was applied through nutritive solutions three times per week. The total quantity of macro-nutriments applied by pot during the experiment, and expressed in mg kg^{-1} of soil in pots with Brachiaria were the followings: 160 of N, 330 of K, 88 of Mg and 150 of S as $Mg(NO_3)_2$, KNO_3, $MgSO_4$ and K_2SO_4; and the total quantity of applied micro-nutriments expressed as mg kg^{-1} of soil were the followings: 54 of Mn as $MnSO_4.H_2O$; 4.0 of Cu as $CuSO_4.5H_2O$; 6.7 of B as H_3BO_3; 2.1 of Mo as $NaMoO_4.2H_2O$; 0.36 of Co as $CoCl_2.6H_2O$; 364 of Fe as $FeCl_3.6H_2O$; 197 of Na as Na_2H_2 EDTA and NaCl. In Stylosanthes were applied: 250 of K, 52 of Mg and 170 of S as $MgSO_4$ and K_2SO_4, and the total quantity of applied micro-nutrients, expressed as mg kg^{-1} of soil were the followings: 55.6 of Mn as $MnSO_4.H_2O$; 3.8 of Cu as $CuSO_4.5H_2O$; 6.9 of B as $H_3BO_{3;}$ 2.2 of Mo as $NaMoO_4.2H_2O$; 0.37 of Co as $CoCl_2.6H_2O$; 374.8 of Fe as $FeCl_3.6H_2O$ and 216 of Na as Na_2H_2 EDTA and NaCl.

Plants were harvested eight weeks after the sow, and were determined aerial dry matter and fresh weight of nodules (in the legume), fresh weight of roots/ soil volume in tubes for isolating rhizosphere and total content of N, P and Ca in tissue. The total N was determined by combustion, using a CHN analyzer (Perkin Elmer 2400 CHN). The Ca and P in tissue were extracted by humid combustion in solution of concentrated $HNO_{3,}$ 33% H_2O_2 and 6N HCl. The Ca and P were determined by ICP (inductively joined plasma). In soil were determined pH, available P and interchangeable Ca in soil of the rhizosphere and non-rhizosphere. The pH was determined on 1:2.5 soil-water relation. The available P was extracted with $NaHCO_3$ 0,5M (Olsen method), and was determined by spectrophotometry using the molybdate blue method (7). The interchangeable Ca was extracted to KCl 1M on a 1:10 soil-extracted solution relation, and was determined by atomic absorption.

The effect of the forage specie on the dissolution of RFs was determined by the difference in available P and interchangeable Ca on each treatment with RF, and the control without P inside each forage specie and lime-coated treatment; these differences will be referred as DP-Olsen y DCa.

Once determined the fresh weight of roots, these were preserved on an ethanol solution at 20%, and were kept in the refrigerator until the moment of measuring them. A computerized system (Scannercomputer) was used with a designed software that measures longitude, thickness and superficial area of roots, based on the discrimination of edges (8) with a 29.5 pixels/cm resolution. In order to improve the scanner contrast, roots were tinged, submerging them on a methylene blue solution at 5% for 5-10 minutes and then were washed with water until remove the excess of color.

A variance analysis of the data was done, using the software for the design of divided smallholdings of the Statistical Analysis System (9), and means test were done by the minimum significant difference (MDS) when F values were significant ($P<0.05$).

Results and discussion

Production of dry matter

There were significant differences ($P<0.05$) in aerial dry matter between forage species, P treatments and their interactions. The average value of aerial dry matter of Brachiaria was the double of the Stylosanthes. In both species, the aerial dry weight average by lime-coated treatment increased with the solubility of the P source (figure 2). There were not significant differences in aerial dry weight between treatments with RFR, RFCN and SFT in Stylosanthes, while for Brachiaria the aerial dry weight was higher with SFT, and reduced in the following order RFCN> RFR >RFMF. With RFMF values of aerial fresh weight were lower than in the control

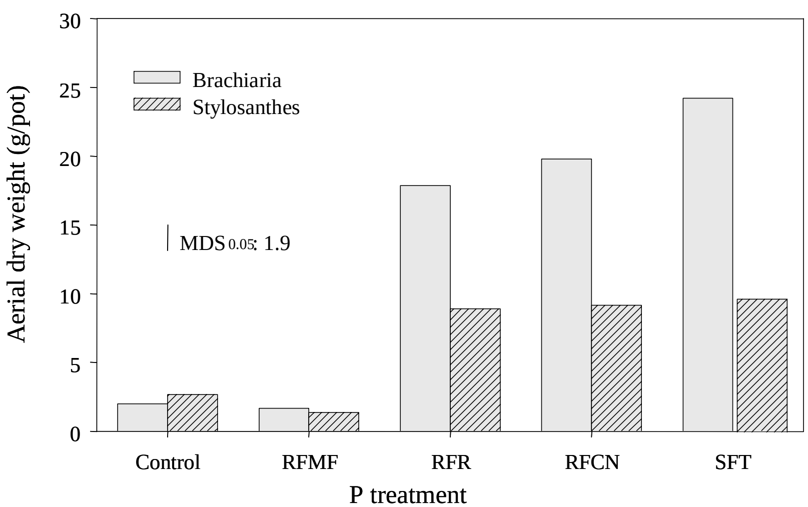

Figure 2. Effect of P treatments on the production of aerial dry matter in Brachiaria and Stylosanthes. Average values of limed treatments.

treatment without P in both forage species.

Absorption of P

The absorbed P by crops was significantly affected by P treatments and it varied between crops. In treatments with RFMF (RF of low solubility) the absorbed P by both crops was similar compare to the control treatment without P, and inferior to the treatments with the other P sources (figure 3). The differences in absorption of P between species were only observed in treatments with SFT, where P adsorption was higher in Brachiaria than in Stylosanthes, which indicates that graminea is more dependent of soluble P sources than legumes. This corresponds to what Van Raij and Van Diest (1), Marschner (6) and Diest *et al*. said (10), who reported that legumes are more likely to use RFs than species that depend on mineral N.

Absorption of Ca

There were significant differences ($P<0.05$) in Ca adsorbed in the forage species, P treatments and the interactions. The average value of Ca adsorbed in Stylosanthes was five times higher than the adsorbed in Brachiaria. Ca absorption in Stylosanthes was significantly higher in treatments with RFCN, RFR and SFT than in treatments with RFMF and the control without P (figure 4). In Brachiaria there were not significant differences in Ca absorption in treatments with P. The average concentration of Ca in Brachiaria was 0.11%, while in Stylosanthes was of 0.16%. It is important to mention that legume acts as a drain of the released Ca from the phosphates, thereby reducing the Ca concentration in soil solution, which can cause the dissolution of RFs. In this sense, Robinson *et al.* (11)

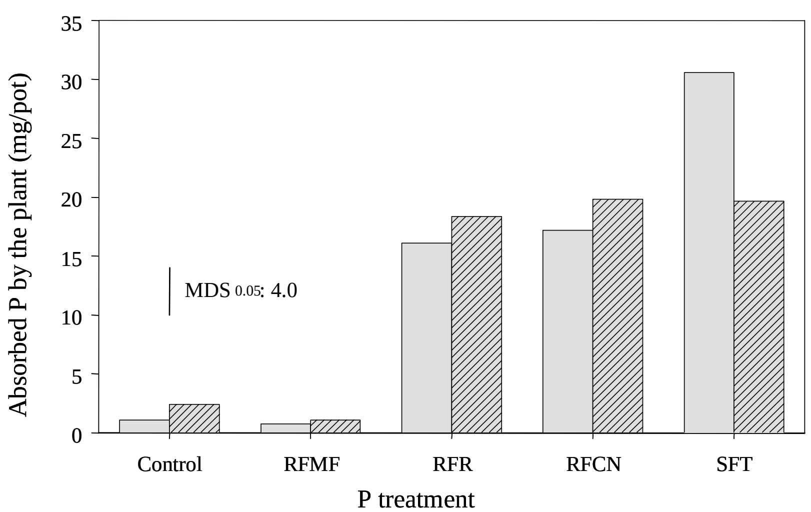

Figure 3. Effect of P treatments on the absorption of P in Brachiara and Stylosanthes. Average values by limed treatments.

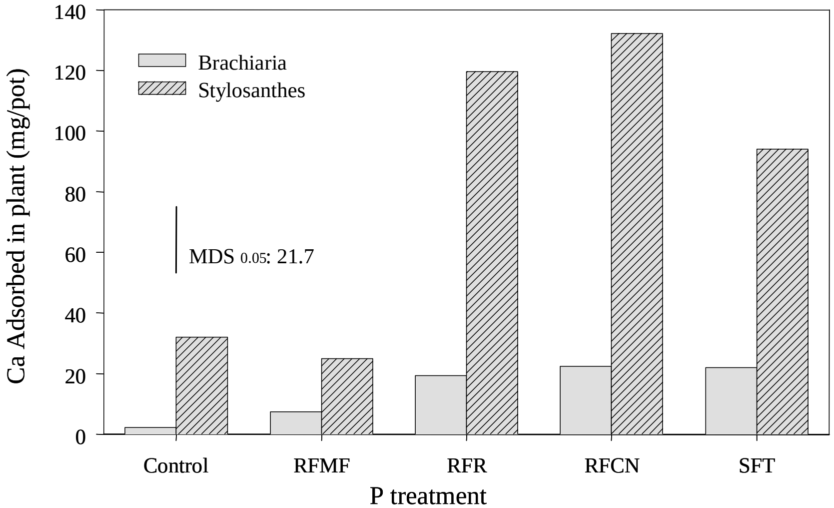

Figure 4. Effect of P treatments on the absorption of Ca in Brachiaria and Stylosanthes. Average values by limed treatments.

reported that RFs dissolution is mostly influenced by the drain's size of Ca, it means, at the same time that increases the adsorption of Ca by the plant, the lower will be its concentration in soil's solution, which favors the RFs dissolution.

Roots development

There were significant differences ($P<0.05$) in longitude, diameter and superficial area of roots (in tubes to isolate soil from the rhizosphere) between species, P treatments and their interactions (table 1). The root's growth in tubes to isolate soil from the rhizosphere was higher in Stylosanthes than in Brachiaria. Brachiaria roots were like fine threads which passed through tubes and developed their root's mass on the inferior division of the pot, meanwhile, Stylosanthes had a lower root's mass on the inferior division of the pot, but did have a higher quantity of ramified roots in tubes and on the superior division of the pot. An average of the total, values of longitude, diameter and superficial area of Stylosanthes's roots on the rhizosphere tubes were 32.2%, 15.6 and 32% respectively higher than in Brachiaria (table 1). The superficial area of roots in both species increased with the solubility of RF, reaching the highest superficial area of roots in treatments with RFCN (table 1). The average value of the superficial area of Stylosanthes's roots in the treatment without P was three times higher than in Brachiaria (table 1). Considering that in this research, legume had a higher absorption of Ca than Brachiaria, and this corresponded to a higher superficial area of roots in the legume, it is therefore possible that these two factors of the plant are indicators of the plant's capacity to use P of the

Table 1. Effect of the specie of the plant and P treatments on the averaged pH values of the soil in the rhizosphere (R), non-rhizosphere (NR), difference between rhizosphere and non-rhizosphere (R-NR) and longitude, diameter and superficial area of Brachiaria and Stylosanthes roots.

Treatment		pH in H_2O			Roots		
Specie	P	R	NR	R-NR	Longitude	Diameter	Superficial area
					--------cm/pot---------		cm^2/pot
Brachiaria	Control	5.79	5.47	0.32	221.5	0.043	10.5
	RFMF	7.48	7.49	-0.02	324	0.047	9.5
	RFR	6.04	5.83	0.21	612.5	0.062	38.0
	RFCN	6.05	5.87	0.19	887.5	0.057	51.5
	SFT	5.49	5.60	-0.11	748.5	0.063	48.5
Average		6.17	6.05	0.12	558.8	0.054	31.5
Stylosanthes	Control	4.71	5.04	-0.33	471.5	0.065	29.5
	RFMF	7.30	7.28	0.02	300.5	0.060	18.0
	RFR	4.54	5.35	-0.82	826.0	0.065	59.0
	RFCN	4.72	5.47	-0.75	1045.5	0.065	66.0
	SFT	4.06	5.09	-1.03	901.5	0.063	57.5
Average		5.06	5.64	-0.58	709.0	0.064	46.0
		---------------------Average of P treatments------------------					
	Control	5.25	5.25	0.00	346	0.054	20
	RFMF	7.39	7.38	0.01	312	0.053	14
	RFR	5.29	5.59	-0.30	738	0.064	48
	RFCN	5.38	5.66	-0.28	966	0.061	59
	SFT	4.77	5.34	-0.57	825	0.063	53
MDS 0.05:							
Specie		0.09	0.07	0.10	122	0.003	6
P		0.14	0.11	0.15	192	0.004	10
P x Especie		0.20	0.15	0.21	181	0.007	14

RFs; these results agree to the indicated by Marschner (6) and Hinsinger and Gilkes (4) when saying that legumes have a higher capacity of using RFs, which causes a higher superficial area of roots, and higher activity of rootlets in legumes.

Plant's effect and P source on the acidification of the rhizosphere

There were significant differences ($P<0.05$) on the pH values of the soil in the rhizosphere and non-rhizosphere due to the main effects and interactions between forages species, lime-coated and P treatment (table 1). RFMF increased significantly the pH of the soil in both crops, where the soil's pH of the rhizosphere of both crops was in a rank between 7.3 – 7 in treatments without being limed. The average values of the soil's pH by forage species indicate that there was a higher acidification on the legume rhizosphere than in the one of the gramineae. The difference on the average value of pH of the rhizosphere and non-rhizosphere soil increased in 0.12 units in pots with Brachiaria, and reduced in 0.58 units in pots with Stylosanthes (table 1). This pH difference between the rhizosphere and non-rhizosphere soil was influenced by the plant's specie as well as by the P source. With the RFMF, the difference in pH was very little for both species. In the other P treatments, the rhizosphere acidification with Stylosanthes in relation to the non-rhizosphere had the following order: SFT > RFR = RFCN > control, while in pots with Brachiaria, the soil's pH of the rhizosphere was higher than in the non-rhizosphere on the control treatments, RFR and RFCN.

Plant's effect on the dissolution of the phosphoric rocks

The RFs dissolution can be estimated through the quantification of the residual RF or the P and Ca quantities in the soil and in the plants (12). In this research, the quantities of Ca and released P from the RFs were estimated with the differences of the interchangeable Ca and available P in the soil between treatments with P and the control at the end of the experiment, and are expressed as DCa y DP-Olsen. There were significant differences ($P<0.05$) in DCa on soil of the rhizosphere, between treatments with P and forage species (figure 5). The high value of DCa for treatments with RFMF in both crops, is associated to the increased in pH of the soil in values of > 7.0 (table 1), which might be due to the release of Ca from the $CaCO_3$ content in RF (13). The application of 50 mg of soluble/Kg P of soil with each P source resulted applying different quantities of Ca, due to the variations on the chemical and mineralogical composition of the used RFs (13). The added quantities of Ca in µg kg^{-1} of soil, with each P source were of 1870 with RFMF, 336 with RFR, 408 with RFCN and 32 with SFT. There were not significant differences in values of DCa between treatments with RFR and RFCN in both crops, however, the quantity of released Ca from these two rocks was higher in Stylosanthes (figure 5). The DCa information for the three RFs corresponds to the acidification results of the rhizosphere (table 1), the production of aerial biomass (figure 2) and the absorption of Ca (figure 4) in crops and in the three RFs. The highest acidification of the rhizosphere in Stylosanthes with RFR and RFCN favored the dissolution of these rocks, which was reflected with a higher development of plants and absorption of Ca than with RFMF or with Brachiaria. The species of the plant

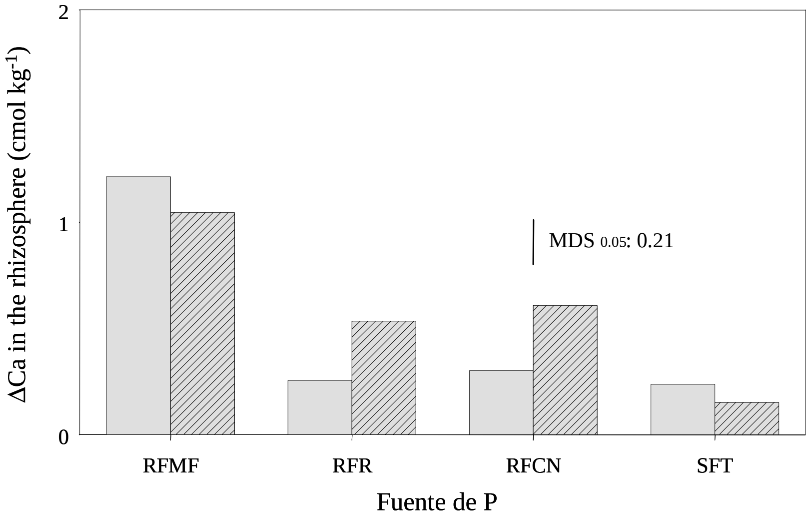

Figure 5. Effect of the plant's specie and P source on the released of Ca by dissolution of phosphoric rocks in the soil of the rhizosphere. Average values by limed treatments.

significantly affected DP-Olsen values of the rhizosphere between P sources (figure 6). The average value of DP-Olsen in the rhizosphere was higher in Stylosanthes than in Brachiaria. The DP-Olsen values in the

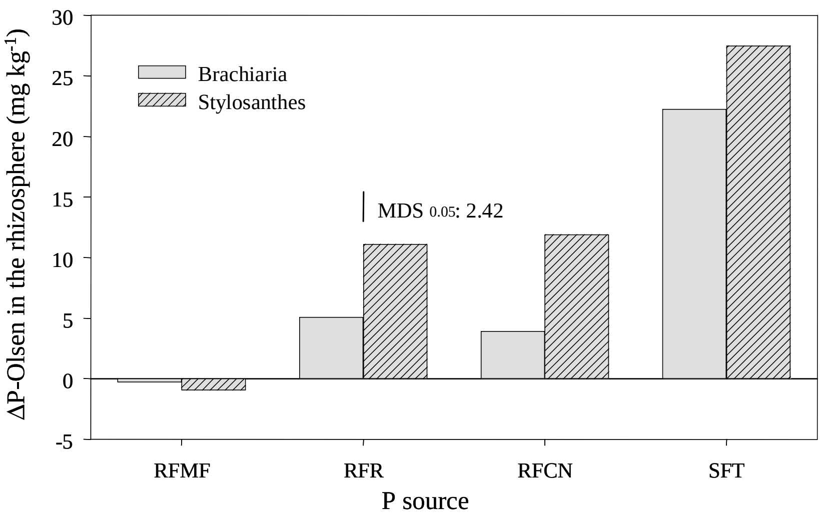

Figure 6. Effect of the plant's specie and P source on the released of P by dissolution of phosphoric rocks in the rhizosphere. Average values by limed treatments.

rhizosphere estimated by forage specie and lime-coated treatment had the following order: TSP > RFR = RFCN > RFMF. The highest values of DP-Olsen with SFT in both crops are consistent with the high solubility of this P source, while DP-Olsen values with RFMF in both crops are consistent to the low solubility of this RF. Likewise, the highest values of DP-Olsen for RFR and RFN with Stylosanthes in relation to Brachiaria are consistent to the highest acidification capacity of the legume, which favors a higher dissolution of RFs and a release of P than in the rhizosphere of the gramineae.

There was a lineal and negative relation between pH and DP-Olsen in the rhizosphere (r = 0.97) for both crops (figure 7), which indicates the importance of the soil's acidity in the dissolution of the RFs. DP-Olsen values increased with the acidity of the rhizosphere. The highest values of DP-Olsen occurred in treatments of RFR and RFCN with Stylosanthes. RFMF had the lowest values of DP-Olsen, which is related to the high content of $CaCO_3$ in this RF (13), the increase in pH (> 7.0) and interchangeable Ca of the soil in treatments with RFMF (table 1 and figure 5), which limited the dissolution of the apatite in this material. These results agree with the theory which expresses those high concentrations of interchangeable Ca in solution, and high pH of the soil limits the dissolution of the phosphoric rocks.

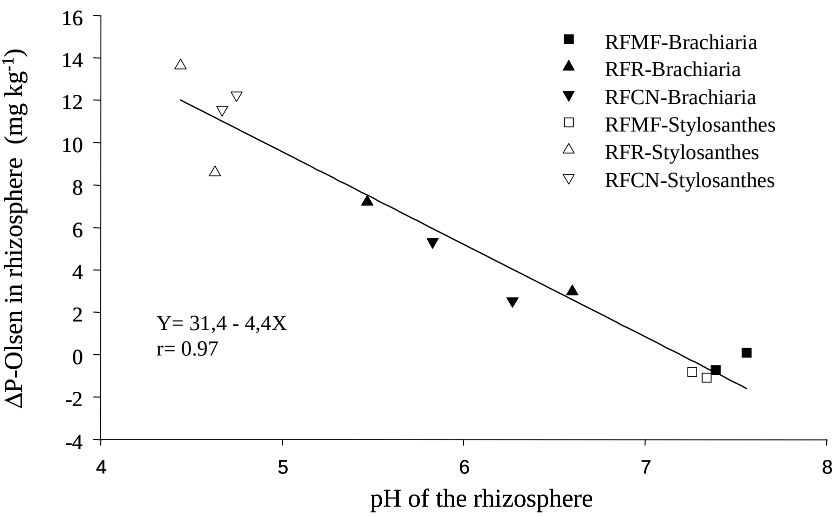

Figure 7. Relation between the pH of the soil of the rhizosphere and released P of phosphoric rocks (ΔP-Olsen) with Brachiaria and with Stylosanthes in limed soil (solid symbols) and soils without limed (empty symbols).

Conclusions

The pH of the rhizosphere reduced with legume (Stylosanthes) and increased with gramineae (Brachiaria). The acidification of the rhizosphere and the higher dissolution of RFs with the Stylosanthes was associated with a higher superficial area of roots, higher absorption of Ca and the dependence of this specie on the biological fixation of N_2, which might have resulted in an excess of cathions in the plant, and the flow of H^+ through the roots to the rhizosphere. The Rfs solubilization was influenced by the interaction of the plant's specie and the mineralogical composition of RFs. The RFMF (RF of low solubility) has a higher content of $CaCO_3$ than RFs, Riecito and North Carolina, which corresponded to the increase in pH (> 7.0) and DCa in soil, and low values of aerial dry matter in both crops. In treatments with RFMF, the rhizosphere acidification by the legume was not enough to promote the dissolution of the apatite in this RF. The RFR behaves much better than RFCN, at the same time that increases the soil's acidity. Further researches are required in order to determine whether the dissolution of RFs and improvements on the availability of P due to the rhizosphere acidification by legumes, as well as was observed on this research, is also applicable in soils with high retention capacity of P.

Literature cited

1. Van Raij, B. and A. Van Diest. 1979. Utilization of phosphate from different sources by six plant species. Plant and Soil 51: 577-589.

2. Aguilar, A. and A.Van Diest.1981. Rock phosphate mobilization induced by the alkaline uptake pattern of legumes utilizing symbiotically fixed nitrogen. Plant and Soil 61: 27-41.

3. Hoffland, E., G.R. Findenegg, and J.A. Nelemans. 1989. Solubilization of rock phosphate by rape. Plant and Soil. 113: 161-165.

4. Hinsinger, P. and R.J. Gilkes. 1995. Root-induced dissolution of phosphate rock in the rhizosphere of Lupins grown in alkaline soil. Aust. J. Soil Res. 33: 477-489.

5. Johnson, J.F., D.L. Allan, C.P. Vance, and G. Weiblen. 1996. Root carbon dioxide fixation by phosphorus-deficient Lupins albus. Plant Physiol. 112: 19-30.

6. Marschner, H. 1995. The soil-root interface (Rhizosphere) in relationship to mineral nutrition. In: Mineral nutrition of higher plants. Academic Press, London, Second Edition.

7. Murphy, J. and J. P. Riley. 1962. A modified single extraction solution method for the determination of phosphate in natural waters. Analytical Chimica Act. 27: 31-36.

8. Pan, W.L. and R.P. Bolton.1991. Root quantification by edge discrimination using a desktop scanner. Agron. J. 83: 1047-1052.

9. SAS PRIMER. 1992. Guide to use SAS System. Statistical Instructional Computing Laboratoty. Department of Statistics, NCSU, Raleigh, NC. 64p.

10. Diest, J., P.G. Marais, R.B.A. Harry, and C.F.H. Heyns. 1971. Relative availability of rock phosphate to different plant species. Agrochemophysica 3: 35-40.

11. Robinson, J.S., J.K. Syers, and N.S. Bolan. 1992. Importance of proton supply and calcium-sink size in the dissolution of phosphate rock materials of different reactivity in soil. J. Soil Sci. 43: 447-459.

12. Bolan, N.S. and M.J. Hedley. 1990. Dissolution of phosphate rocks in soils. 2. Effect of pH on dissolution and plant availability of phosphate rock in soil with pH dependent charge. Fert. Res. 24: 125-134.

13. Pérez, M. J. 2000. Comparative effect of ion uptake and root growth patterns of forage legume and grass on rhizosphere acidification and solubilization of phosphate rocks. Tesis Doctoral. Universidad del Estado de Carolina del Norte, Raleigh, NC, USA. 123p.

14. Khasawneh, F.E and E.C. Doll. 1978. The use of phosphate rock for direct application to soil. Adv. in Agron. 30: 159-204.