# PHOSPHORUS-MOLYBDENUM RELATIONSHIP IN SOIL AND RED CLOVER (*Trifolium pratense* L.) ON AN ACID ANDISOL

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**ABSTRACT**

We studied the phosphorous (P) and molybdenum (Mo) relationship in soil and red clover (*Trifolium pratense* L.) in a non limed and limed acid Andisol of Southern Chile. In soil, we evaluated the effect of different liming (0 and 2000 mg $kg^{-1}$), P (0, 200 and 400 mg $kg^{-1}$), and Mo (0, 0.58 and 0.96 mg $kg^{-1}$) doses supply on soil available Mo. In addition, the availability of P and Calcium (Ca) in treated soils was determinated. In red clover, we studied the Mo and P shoot concentrations and dry matter yield in response to the different treatments applied to the soil. Also, we measured the changes produced by Mo uptake in shoot Cu concentrations. The results showed that both, lime and more strongly P and Mo additions significantly ($P \leq 0.05$) increased soil Mo availability. In contrast, soil available P was not significantly ($P \leq 0.05$) affected by liming and Mo treatments. A significant high correlation (r = 0.579, at $P \leq 0.05$) was observed among soil Mo availability and shoot Mo concentrations, as well as between soil available P and shoot concentration of P (r = 0.844, at $P \leq 0.01$). In this study for all fertilization treatments, shoot Cu concentrations reached values which are considered as normal for forage species. We also shown that the simultaneous applications of high P and Mo rates could be produce red clover shoot Cu/Mo ratios that should provoke Mo-induced Cu deficiency (Molybdenosis) for the cattle. Red clover yield was no significantly different in limed and non limed soils. Nevertheless, red clover yield production increased at increasing rates of P and Mo in both, non limed and limed soil. The major practical implication of these results is that the application of Mo doses equal or superior to 200 g $ha^{-1}$ to acid Andisols, are recommendable to obtain appropriate Mo shoot content on red clover. In addition, our results shown that P supply to these soils, rather than liming, is necessary to obtain sufficient values of shoot Mo concentrations in red clover.

**Keywords:** Phosphorous, Molybdenum, Andisol, Red clover.

## INTRODUCCION

Red clover (*Trifolium pratense* L.) is a forage legume widely used in the Southern Chile due its high yield and good nutritional properties (Ortega *et al*., 1998). Nevertheless, in Chilean Andisols in which large areas of red clover pastures are established both, acidic conditions (Mora *et al*., 2002; 2006) and high anion retention capacity (Huang and Violante, 1986) affects the availability of some nutrients, such as phosphorous (P) (Bolan *et al*. 2003, Barrow *et al*., 2005; Mora *et al*., 2006) and molybdenum (Mo) (Haynes, 1983, 1985; López *et al*., 2007; Vistoso *et al*., 2005, 2009).

Soil conditions required for adequate yield and quality levels of red clover includes high fertilizer inputs and soil pH values above 5.5 (Mora *et al.*, 2005; López *et al*., 2007). Therefore, soil acidity, as well as P and Mo deficiencies are recognized as limiting factors for red clover production in these soils (Mora *et al*., 2005). Specifically for Mo, it primer role in legume species is it involvement in the symbiotic process of nitrogen (N) fixation (Marschner, 1995).

According previous studies (Zhang and Sparks, 1989; Vistoso *et al*., 2009), P strongly compete with Mo by active sites increasing Mo availability in acidic Andisols. In fact, Haynes (1983) and Bolan *et al.* (2003) indicated that soil Mo availability for plants grown in acid soils depends largely on the extent to which phosphate anions form slowly-soluble complexes or is adsorbed onto mineral surfaces, which are strongly determined by soil pH. Vistoso *et al*. (2009) reported that in Chilean Andisols phosphate is adsorbed strongly in Fe- and Al- humus complex, whereas molybdate is adsorbed especially in iron oxides. Thus, Mo availability for plant uptake in acidic soils is dependent of soil pH, decreasing its adsorption when soil Fe and Al oxides increased (Vistoso *et al*., 2009). Parfitt (1978) indicated that specific adsorption of phosphate and molybdate by soil colloids decreased as the pH is increased. Indeed, we earlier results (López *et al*., 2007), in agreement with those reported by Bolan *et al*. (2003), have shown that Mo availability is increased in response to lime and P supply.

On the other hand, we preliminary soil incubation and greenhouse studies (López *et al*., 2007) showed that soil available Mo increased with Mo fertilization. Moreover, López *et al*. (2007) found a positive correlation between both, red clover yield and shoot Mo concentrations with soil Mo availability in Chilean Andisols.

Jarrel *et al*. (1980) reported that Mo toxicity in animal diet is dependent, between other parameters, of cupper (Cu) concentration in the same one. Thus, soil Cu supply has been shown reduced Mo toxicity. In this way, Molybdenosis is an animal diet disorder defined as Mo-induced Cu deficiency (Suttle, 1991). According to O'Dell (1997), the best indicator of this animal disease is the Cu/Mo ratio in animal diet. In the same hand, Whitehead (2000) indicated critical values for Cu/Mo ratio in animal foods of 2.0, indicating that lower ratios could be provoke Cu deficiency for the cattle.

The aim of this study was to evaluate the P and Mo relationship in an acid Andisol of Southern Chile and its effect on Mo availability and red clover yield and mineral nutrition under greenhouse conditions.

## MATERIALS AND METHODS

### Greenhouse experiment

The experiment was conducted in pots on greenhouse conditions using an Andisol soil (Piedras Negras Series of Southern Chile) never amended with Mo fertilizer. A completely randomized 2 x 3 x 3 factorial design was applied.

Soil samples were collected from a natural pasture at top 20 cm. Some of the soil chemical properties are shown in Table 1. The chemical composition of the soil was determined according to the

**Table 1.** Some chemical properties of the soil studied.

| **Parameters** | **Soil content** |
|---|---|
| pH $H_2O$ | 5.26 |
| Organic matter (%) | 18.00 |
| Organic C (%) | 10.50 |
| Olsen P (mg $kg^{-1}$) | 2.00 |
| K ($cmol_c$ $kg^{-1}$) | 0.13 |
| Na ($cmol_c$ $kg^{-1}$) | 0.05 |
| Ca ($cmol_c$ $kg^{-1}$) | 0.73 |
| Mg ($cmol_c$ $kg^{-1}$) | 0.30 |
| Al ($cmol_c$ $kg^{-1}$) | 0.17 |
| Al saturation % | 12.32 |
| Al Ext. (mg $kg^{-1}$) | 2097 |
| Cu (mg $kg^{-1}$) | 0.94 |
| Mo (µg $kg^{-1}$) | 23 |

methodology described earlier by Sadzawka *et al.* (2004).

Soil samples (1.1 kg $pot^{-1}$) were weighed. Lime ($CaCO_3$) was added to half of total pots at final concentrations of 2000 mg $kg^{-1}$ soil. The lime dose was selected based on previous studies of our research group (Lopez *et al*., 2007) and applied according to the incubation method described by Mora and Barrow (1996).

Both, non limed and limed samples were fertilized with 0, 200, or 400 mg P $kg^{-1}$ soil (as $P_2O_4$, Triple Super Phosphate) to render six combinations of acidity and P content in the soil.

The Mo-pelleted red clover seeds (as ammonium molybdate, 54.31 % of Mo) were sown in each pot. Three doses of Mo were tested (0, 0.58, and 0.96 mg Mo $kg^{-1}$ soil, equivalent to 0, 200, and 400 g Mo $ha^{-1}$ soil as $Na_2MoO_4$).

All treated samples were also fertilized with 40 mg sulphur (S) $kg^{-1}$ soil (as Sulpomag) and a solution of Boron was applied to each pot at plant emergence state (25 post-sown days).

In every experiment, 50 seeds of red clover (*T. pratense* cv. Toltén) per pot were sown, and after germination plants were thinned to 20 seedlings per pot. Three pots were used as replicates for each treatment, and the pots were periodically repositioned. During the growth period the plants were watered daily with distilled water and one cut was harvested for chemical analysis at 25 cm plant height.

**Soil chemical analysis**

The soil chemical analyzed according Sadzawka *et al*. (2004). P was extracted by the Olsen bicarbonate method and analyzed by the Murphy and Riley method as described previously (1962). Ca was extracted with a diethylene triaminepentaacetic acid (DTPA)-$CaCl_2$-tiethanolamine (TEA) solution (pH 7.3) and analyzed by Flame Atomic Absorption Spectrophotometry (FAAS).

The available Mo was determined by extracting 10 g of soil with a solution of 20 ml of ammonium bicarbonate (AB)-DTPA (Soltanpour *et al*., 1982). The suspension was shaken for 15 min, filtered, and the available Mo was determined by Graphite-Furnace (GF 90) Atomic Adsorption Spectrophotometer, UNICAM 960. Soil pH was measured by potentiometry in a 1:2.5 (w/v) soil/distilled water suspension. All determinations were carried out at the end of the assay, after the red clover harvest.

**Plant chemical analysis**

Shoots was weighed and the samples was dried at 65ºC for 48 h to determine both dry matter (DM) and foliar mineral concentrations. For P shoot concentration the molybdo-vanadate method as described by Sadzawka *et al*. (2007) was used. The calcium (Ca) and Cu shoot concentrations were determined by Atomic Absorption Spectrophotometry (AAS), after that dry plant samples were ashed at 500°C for 4-8 h and digested with a acid mixture composed by $HNO_3$-HCl and $H_2O$ (Sadzawka *et al*. 2007). The Mo chemical analysis was carried out using AAS coupled to a Graphite-Furnace (AAS-GF), after that dry shoot samples were ashed at 500°C for 4-8 h, dissolved in diluted HCl, and filtered (Kalra, 1998).

**Statistical analysis**

Data were assessed by two-way ANOVA using SigmaStat 3.1 software. Means were compared using Tukey's test ($P \leq 0.05$). The effect of fertilization treatments in non limed and limed soil was analyzed separately. Pearson r correlation ($P \leq 0.01$ and $P \leq 0.05$) was calculated and used to test the relationships between two response variables (JMP version 5.0.1 statistical program, SAS Institute).

## RESULTS AND DISCUSSION

**Soil nutrients availability**

The results of pH values and the soil Ca, P, and Mo availability in non limed and limed soil at different P and Mo doses are showed in Table 2. In agreement with previous works (Haynes, 1985; Bolan *et al*. 2003; Lopez *et al*. 2007), the results indicated that soil available Mo increased slightly in response to liming but strongly with Mo and P doses supplied. According to Bolan *et al*. (2003) and Goldberg *et al*. (2002), lime application substantially increased the availability of native Mo in soils. Mora and Barrow (1996) agree that lime increases the soil pH, then the negative charge of soil components also increase, which can promote anion desorption. In fact, Goldberg *et al*. (1996) observed that the maximum Mo adsorption on amorphous iron (Fe) and aluminum (Al) oxides minerals occur at soil pH from 4.0 to 5.0, decreasing at increasing pH. Besides, Vistoso *et al*. (2009) shown that in Chilean Andisols the adsorption of molybdate and phosphate decreased as pH values are increased, detecting adsorption levels of 100% for both anions at pH 4.0. The results of the same work also indicated that with pH increases of 5.0 to 6.0, the adsorption of Mo diminished from 78% to 66%. These effects may be particularly important in south Chilean Andisols, in which liming is frequently accompanied by high P additions (Mora *et al*., 1999; 2002). Barrow *et al*. (2005) report that both, P and Mo adsorption are described by ligand exchange mechanism and then an increase in hydroxyl ions could be made both anions more available. Many sites with high affinity for molybdate in soils may be blocked by phosphate anions (Goldberg *et al*. 2002). Phosphate anion competes strongly with molybdate for active site in Chilean Andisols (Vistoso *et al*., 2005) and this capacity decreased at increasing pH (Barrow, 1986; Xie and MacKenzie, 1991; Vistoso *et al*., 2009).

In this study, soil pH values increased by the effect of lime application from averages of 5.33 to 5.56. Furthermore, we observed a significant high correlation (r = 0.930, at $P \leq 0.01$) between soil Ca availability and soil pH values (Figure 1). It is noteworthy that in acid soils available Ca is very low. When soil pH increase by liming, then an increment of negative charges is provoked and the cationic interchange capacity of soils is increased. This allows to infer that a large amount of Ca applied is retained by new interchange sites (Galindo and Escudey, 1985).

Our results showed that the higher value of available Mo in non limed soil (2150 $\mu g\ kg^{-1}$ soil) was obtained with the highest P and Mo rates. However, in

**Table 2.** Effect of P and Mo additions on soil pH and Ca, P, and Mo availability in non limed and limed soil at the end of greenhouse experiment.

| **Fertilization doses** | | | **Soil available nutrients** | | |
|---|---|---|---|---|---|
| **P ($mg\ kg^{-1}$)** | **Mo ($g\ ha^{-1}$)** | **Soil pH** | **Ca ($cmol_c\ kg^{-1}$)** | **P ($mg\ kg^{-1}$)** | **Mo ($\mu g\ kg^{-1}$)** |
| | | **Non limed soil** | | | |
| **0** | **0** | 5.26 | 3.09 **b** | 9.00 **c** | 22 **e** |
| | **200** | 5.29 | 3.08 **b** | 9.00 **c** | 551 **d** |
| | **400** | 5.31 | 3.11 **b** | 9.67 **c** | 1335 **c** |
| **200** | **0** | 5.33 | 3.53 **ab** | 21.67 **b** | 24 **e** |
| | **200** | 5.37 | 3.45 **ab** | 20.00 **b** | 797 **d** |
| | **400** | 5.39 | 3.52 **ab** | 20.33 **b** | 1719 **b** |
| **400** | **0** | 5.36 | 3.67 **a** | 29.00 **a** | 28 **e** |
| | **200** | 5.36 | 3.79 **a** | 33.33 **a** | 817 **d** |
| | **400** | 5.33 | 3.84 **a** | 33.67 **a** | 2150 **a** |
| | | **Limed soil** | | | |
| **0** | **0** | 5.61 | 6.58 **b** | 8.67 **c** | 27 **f** |
| | **200** | 5.59 | 6.47 **b** | 8.67 **c** | 285 **e** |
| | **400** | 5.61 | 6.49 **b** | 8.67 **c** | 892 **c** |
| **200** | **0** | 5.57 | 6.75 **ab** | 20.00 **b** | 31 **f** |
| | **200** | 5.52 | 7.01 **ab** | 22.67 **b** | 678 **d** |
| | **400** | 5.52 | 6.84 **ab** | 23.00 **b** | 1971 **a** |
| **400** | **0** | 5.52 | 7.10 **a** | 31.00 **a** | 32 **f** |
| | **200** | 5.54 | 7.12 **a** | 31.00 **a** | 985 **c** |
| | **400** | 5.57 | 7.10 **a** | 31.33 **a** | 1475 **b** |

Means with different letters show significant differences between treatments according to the Tukey test ($P \leq 0.05$). The effect of fertilization treatments in non limed and limed soil was analyzed separately.

limed soil supplied with 200 mg $kg^{-1}$ of P and 400 g Mo $ha^{-1}$, available Mo (1971 $\mu g\ kg^{-1}$) was higher than those detected with the same Mo doses and 400 mg $kg^{-1}$ of P (1475 $\mu g\ kg^{-1}$).

As expected, we found that soil available P increased at increasing P rates applied in both, limed and non limed soil. Thus, soil available P ranged from 8 - 9 mg $kg^{-1}$ (control plants) to 20 - 33 mg $kg^{-1}$ when P was applied. The levels of available P were similar in non limed and limed soil, irrespective to Mo doses applied (Table 2).

Regarding liming effects on soil available P in Andisols, conflicting views are held. Indeed, lime has been reported to increase or decrease P adsorption in different soils (Haynes, 1985) and even within the same soil type (Haynes, 1983).

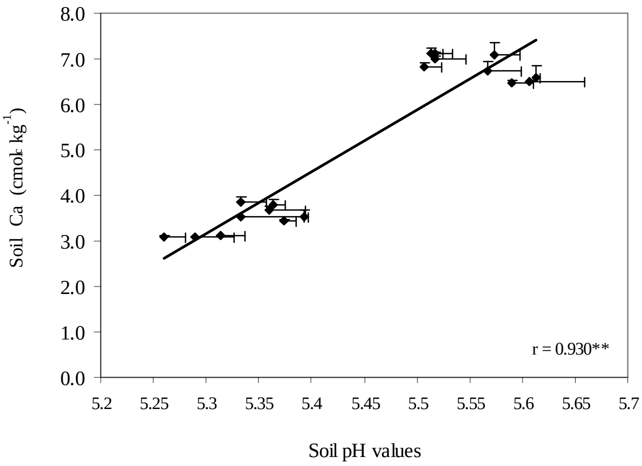


**Figure 1.** Correlation (Pearson's) level among soil available Ca and different pH values in the soil studied. The Pearson correlation was used to test the relationships between the response variables. Asterisks denote significance (*$P \leq 0.05$, **$P \leq 0.01$). Available Ca and soil pH values of non limed and limed soil were included for the analysis.

Mora *et al*. (1999) demonstrated that lime addition on acid south Chilean Andisols reduces P adsorption capacity. They also shown that lime supply on soils with P high initial levels diminishes soil available P. While, when lime was apply on soils with low available P, the amendment cause an increase of soil P availability.

According to Naidu *et al*. (1990), when soil pH increase until values over 6.0 in response to liming, then soil available P should be decrease as a result of insoluble Ca-P complex formation. Besides, lime supply increases Ca concentration on charged soil surfaces turn the potential of the adsorption plane less negative, increasing P adsorption capacity (Barrow, 1980). However, in this study we suggest that the effect of both, red clover rhizosphere and P uptake simultaneous mechanisms, did not allow to detect differences for soil available P between limed and non limed soils.

Previously, López *et al*. (2007) found that Mo additions of 0.58 mg $kg^{-1}$ soil (equivalent to 200 g Mo $ha^{-1}$) to limed and P fertilized (200 mg P $kg^{-1}$) acid Andisols significantly increased available Mo up to 200 µg $kg^{-1}$ soil. These results are in agreement with the data obtained in the present study. Whitehead (2000) indicated that normal values of Mo content in soils ranged from 100 to 4000 µg $kg^{-1}$ soil.

## Shoot mineral concentration and dry matter production of red clover

The effect of fertilizer treatments on Mo and P red clover shoot concentrations are shown in Figure 2. In agreement with previous results (Vistoso, 2005; Lopez *et al*., 2007), we found that Mo uptake increased in response to liming and P supply (Figure 2).

It is known that lime additions to acid soils increases the root growth of legume forages, permitting a greater nutrient uptake by plants (Brauer *et al*. 2002). In addition, as we indicated above, our data showed that Mo availability in limed and P fertilized soils was higher than available Mo in untreated soils. These antecedents can be explain the high values of shoot

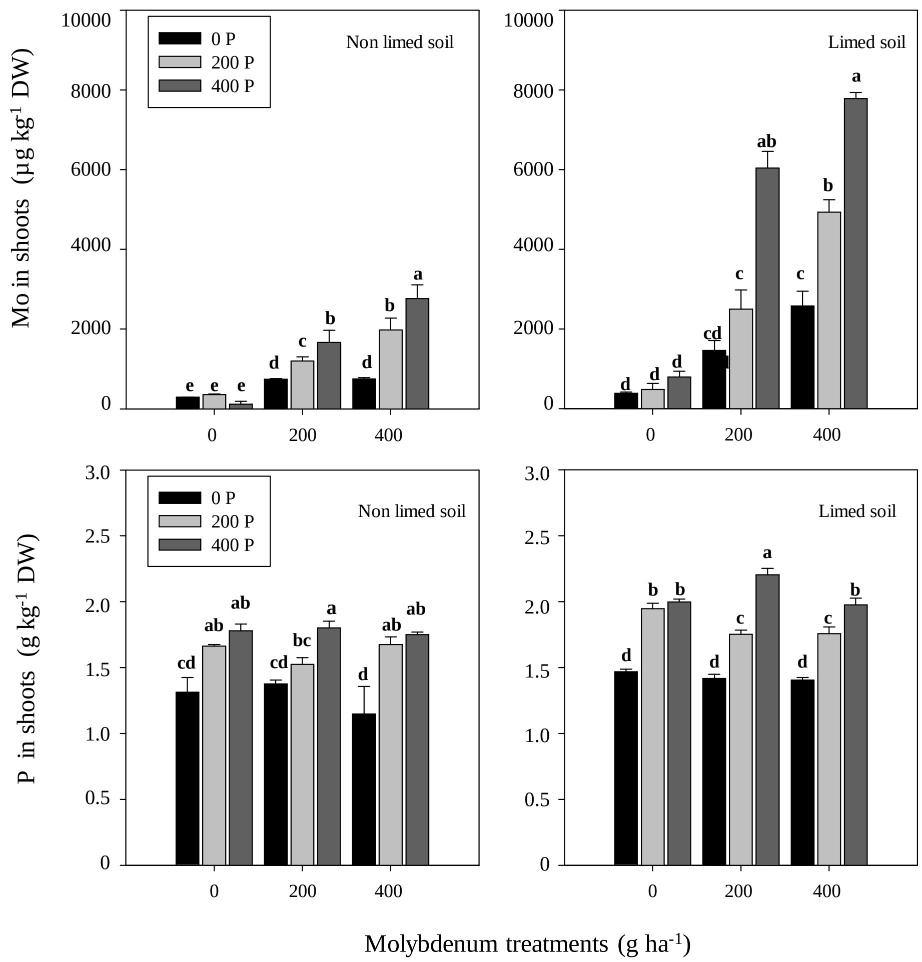


**Figure 2.** Shoot P and Mo concentrations on red clover grown in non limed and limed soil at different P and Mo additions. Means with different letters show significant different between treatments according to the Tukey test ($P \leq 0.05$). The effect of fertilization treatments in non limed and limed soil was analyzed separately.

Mo detected here in plants grown in soil supplied with lime and P.

Moreover, we found that shoot Mo concentrations in red clover increased as the level of Mo rates applied was increased both, in non limed and limed soil. Similar results have been reported previously by Adams (1997), Vistoso (2005) and Lopez *et al*. (2007). In fact, our results indicated that Mo concentration in shoots was significantly correlated (r = 0.579; $P \leq 0.05$) with Mo availability in the soil (Figure 3a).

Shoot Mo concentration in red clover plants untreated with Mo and P was 0.29 mg kg$^{-1}$ DM in non limed soil and 0.39 mg kg$^{-1}$ DM in limed soil. Then, the addition of Mo doses equivalent to 400 g ha$^{-1}$ plus 200 mg kg$^{-1}$ of P to non limed soil increased Mo shoot concentrations to 1980 µg Mo kg$^{-1}$ DM, and to 2770 µg Mo kg$^{-1}$ DM when 400 mg kg$^{-1}$ of P were applied. Furthermore, in limed soil the effect of P doses on shoot Mo concentration was higher than those detected in non limed soil. With P supply of 200 and 400 mg P kg$^{-1}$ in limed soil, shoot Mo concentrations increased until to 4930 and 7780 µg kg$^{-1}$, respectively. Bennet (1993), reported critical, sufficient, and toxic levels indexes of Mo in plant shoots ranges from 0 to 100 µg kg$^{-1}$ DM, from 100 to 500 µg kg$^{-1}$ DM, and from 10000 to 50000 µg kg$^{-1}$ DM. However, Lopez *et al*. (2007) determined that the critical Mo concentration deficiency value for red clover grown in the soil studied here was 500 µg kg$^{-1}$ of DM. Accordingly, Shuman (1994) reports that the critical concentration of Mo for red clover plants ranged between 20 to 490 µg kg$^{-1}$ DM, while values from 500 to 1000 µg kg$^{-1}$ DM are defined as appropriate levels.

It is important mentioned that normal levels of Mo concentration for animal diet are values lower than 5000 µg kg$^{-1}$ DW. In USA, Canada, New Zealand, and Switzerland, toxic effects of Mo foliar concentration have been found on forage species containing between 15 to 300 mg Mo kg$^{-1}$ DM (Adriano, 2001).

Taking these antecedents as a basis, we suggest that the simultaneous application of Mo supply and high doses of P to limed south Chilean Andisols can be provoke toxic levels of Mo content on red clover for the cattle. Therefore, irrespective of lime and P supplies, we demonstrated that soil Mo additions of 200 g ha$^{-1}$ should be sufficient to reach normal levels of Mo in plants (up to 500 µg kg$^{-1}$ of DW).

On the other hand, our results indicated that shoot P concentrations of red clover plants grown in limed soil increased respect to those grown in non limed soils (Figure 2). These results are in agree with previous reports (Vistoso, 2005; Mora *et al*., 2002). Also and as expected, we found that the application of increasing P rates increased steadily shoot P concentration according to Jones *et al*. (1980) and Basak *et al*. (1982). Shoot P concentrations were positively correlated ($r = 0.844$, $P \leq 0.01$) with soil available P levels (Figure 3b). In addition, we demonstrated that Mo supply do not affected significantly P uptake (Figure 2).

In this study, P concentration in shoots ranged from 1.15 to 2.20 g kg$^{-1}$ DM. The highest value of shoot P (2.20 g kg$^{-1}$ DM) was observed in plants grown in limed soil treated with 400 mg P kg$^{-1}$ and 200 g Mo ha$^{-1}$. In this hand, Jones *et al*. (1980) reported normal values of shoot concentration of P between 2.0 to 2.8 g kg$^{-1}$ DM, whereas Bolland *et al*. (1995) indicated normal levels ranged from 2.0 to 3.0 g kg$^{-1}$ DM. These P concentrations values are similar to those reported as normal P shoot contents in others legumes forage, such as subterraneum clover (*Trifolium subterraneum* L.) with 2.25 g kg$^{-1}$ DW (Drlica and Jackson, 1979) and white clover (*Trifolium repens* L.) with 3.50 g kg$^{-1}$ DM (Sinclair *et al*., 1997).

In the present study, we found that shoot Cu concentrations of red clover in response to fertilization treatments was negatively correlated with Mo availability in the soil ($r = 0.631$, at $P \leq 0.01$) (Figure 4). Indeed, the highest shoot Cu levels were observed in control plants grown in non limed soil (12.47 mg Cu kg$^{-1}$ DM, *data not shown*). These values were decreasing at increasing Mo as well as when P doses was increased, reaching 6.99 mg Cu kg$^{-1}$ DM for plants grown under the treatment of 400 g Mo ha$^{-1}$ plus P supplies of 400 mg kg$^{-1}$ (*data not shown*). In limed soil, a similar tendency was observed. Nevertheless, in our study shoot Cu concentrations ranged inside

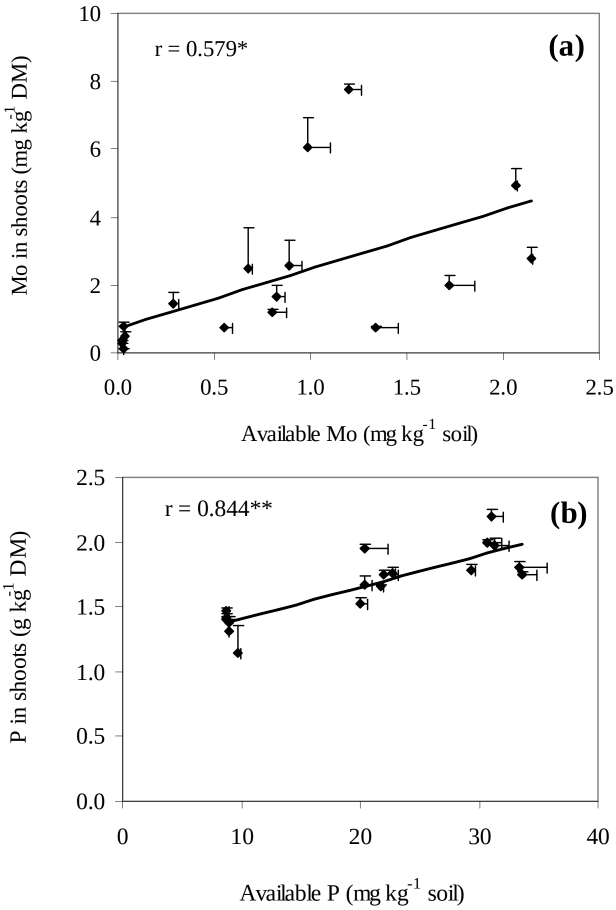


**Figure 3.** Correlation levels among soil P availability and shoot P concentrations (a) and among soil available Mo and shoot Mo concentrations (b) in red clover plants grown in the soil studied. The Pearson correlation was used to test the relationships between the response variables. Asterisks denote significance (*$P \leq 0.05$, **$P \leq 0.01$). Both, data obtained for non limed and limed soil were included for the analysis.

normal values for forage species according Mitchell *et al*. (1957) and Kabata-Pendias and Pendias (2000). Moreover, we detected shoot Cu/Mo ratios from 1.07 to 58.49 (Table 3). Whitehead (2000) reports critical Cu/Mo ratios in animal foods of 2.0, indicating that lower ratios could be provoke Cu deficiency (Molybdenosis) for the cattle. According to these reports, we also demonstrated that the addition of P and Mo high fertilizers rates to acid Southern Chilean Andisols could be produce red clover shoot Cu/Mo ratios that should be induce Cu deficiency. This aspect is especially important in limed soil, in which soil available P and Mo are higher than in non limed soil.

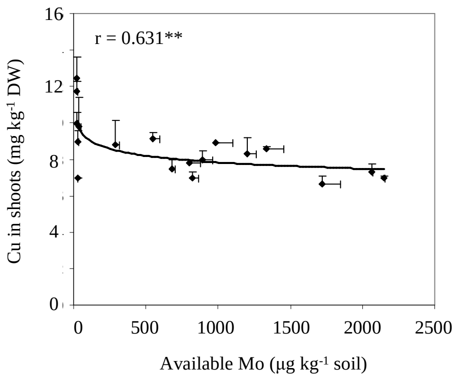


**Figure 4.** Correlation level among soil available Mo and shoot Cu concentration of red clover grown in the soil studied. Significant at **0.01 probability level. Data obtained for non limed and limed soil were included for the analysis.

**Table 3.** Cu/Mo ratios on red clover shoots treated with different P and Mo addition in non limed and limed soil.

| Fertilization Doses | | Shoot Cu/Mo ratios | |
|---|---|---|---|
| **P (mg kg$^{-1}$)** | **Mo (g ha$^{-1}$)** | **Non limed soil** | **Limed soil** |
| 0 | 0 | 43.32 | 25.74 |
| | 200 | 12.32 | 6.65 |
| | 400 | 11.48 | 3.94 |
| 200 | 0 | 32.79 | 24.18 |
| | 200 | 6.60 | 4.20 |
| | 400 | 3.53 | 1.53 |
| 400 | 0 | 58.49 | 11.77 |
| | 200 | 4.45 | 1.53 |
| | 400 | 2.61 | 1.07 |

We shown that red clover DM production in limed soil was similar or higher than those observed for plants grown in non limed soils (Table 4). Demanet *et al.* (1999) reported that red clover is a forage specie tolerant to soil acidity conditions, specially adapted to acid soils of Southern Chile. On the other hand and in agreement with Holford (1985) and Vistoso (2005), we found that shoot DM production was increased at P increasing rates in both, limed and non limed soil. These increases reached until 25% in limed soil with the highest P doses. Respect to the effect of Mo supply on red clover yield, in non limed soil DM production was increased when soil Mo rates was increased. These increments were higher in the presence of P treatments (until 17%). Similarly, in limed soil, the red clover yield was increased with Mo addition, except for the treatment with the simultaneous application of the highest P and Mo doses, also reaching increases of about 17% respect to the control.

López *et al.* (2007) demonstrated that red clover DM production was increased at increasing doses of Mo applied to four South Chilean Andisols. According the results of the same work, yield was stabilized when 0.46 mg Mo kg$^{-1}$ (equivalent to 160 g Mo ha$^{-1}$) were applied. Wheeler (1998), observed that the production of white clover in the first year increased in response to the application of 150 g Mo ha$^{-1}$. Moreover, Vistoso (2005) reported that the highest DM production level of white clover was obtained in limed South Chilean Andisols with P rates of 100 to 200 mg P kg$^{-1}$ soil and Mo doses of 4 to 6 mg Mo kg$^{-1}$ soil.

We also found that in non limed soil the highest red clover production was detected for plants treated with the highest P and Mo doses (6.70 g pot$^{-1}$), increased 28% higher compared with control plants (4.80 g pot$^{-1}$). While, the maximum production in limed soil was obtained with 400 mg P kg$^{-1}$ soil and 200 g Mo ha$^{-1}$ soil (6.87 g pot$^{-1}$), being this value 30% higher than the DM production of control plants (Table 4).

According to our results, lime supplies to the soil studied not affected importantly red clover DM production of red clover, provided that heavy P doses are applied. Demanet *et al.*, (1999) indicated that red clover can resist high soil acidity showing high DM yields.

**Table 4.** Shoot dry matter (DM) production of Red clover at different P and Mo additions in non limed and limed soil.

| Fertilization Doses | | Dry Matter (g pot$^{-1}$) | |
|---|---|---|---|
| **P (mg kg$^{-1}$)** | **Mo (g ha$^{-1}$)** | **Non limed soil** | **Limed soil** |
| **0** | **0** | 4.80 **e** | 5.16 **d** |
| | **200** | 4.78 **e** | 5.52 **cd** |
| | **400** | 4.97 **de** | 5.69 **cd** |
| **200** | **0** | 5.46 **cd** | 5.12 **d** |
| | **200** | 6.20 **b** | 6.04 **bc** |
| | **400** | 6.32 **b** | 5.97 **bc** |
| **400** | **0** | 5.75 **bc** | 6.47 **ab** |
| | **200** | 6.18 **b** | 6.87 **a** |
| | **400** | 6.70 **a** | 6.42 **ab** |

Means with different letters show significant differences between treatments according to the Tukey test ($P \leq 0.05$). The effect of fertilization treatments on dry matter production in non limed and limed soil was analyzed separately.

According to the same study, this forage legume is adapted to south Chilean acid soils, requiring only a few amounts of lime for its successful growth. They agree that lime supply of 1 ton ha$^{-1}$ (with $P_2O_4$ rates of 180 kg ha$^{-1}$) are sufficient to reach the maximum yield of red clover pastures. Moreover, they also had shown that the yield responses of red clover to lime additions are detectable only at last productive season.

## CONCLUSIONS

The results presented in this paper shown that lime and P applications to south acid Chilean Andisols significantly increased soil available Mo and shoot Mo concentration in red clover plants. Nevertheless, we found that liming and Mo supply did not affected soil P availability. In addition, our results confirm previous reports indicating that P heavy doses supply are necessary to reach sufficient levels of shoot P concentration of red clover grown in acid Andisols and that P fertilization is more important for red clover yield than liming in these soils.

Furthermore, we had shown that the additions of Mo doses equivalent to 200 g ha$^{-1}$ and 200 mg P kg$^{-1}$ soil are recommendable to obtain appropriate Mo shoot content in pastures of red clover grown in limed Andisols.

Also, under this fertilization strategy proposed, shoot Cu foliar concentrations ranged values which are considered normal levels for forage legume species. We also found that the applications of high P and Mo supply simultaneously, could be produce red clover shoot Cu/Mo ratios that should be cause Cu deficiency for the cattle.

## ACKNOWLEDGEMENTS

This work was supported by the International Cooperation FONDECYT project 7060093 and by the FONDECYT project 1061262 and Grants.

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