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Occurrence and effect of arbuscular mycorrhizal propagules in wheat as affected by the source and amount of phosphorus fertilizer and fungal inoculation

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Abstract

Volcanic ash-derived soils in Chile show very unique behavior and properties as soil system due to their unusual composition characterized by high allophane and stabilized humus content. These soils constitute excellent models to study both natural and man-induced VA mycorrhizal effect over plant nutrition and soil ecology sustainability. This paper studies the effect of *Glomus etunicatum* inoculation of wheat in a natural volcanic soil fertilized with soluble P or with partially acidulated-rock phosphate (pa-RP) at two rates (17 and 86 kg P ha^{-1}); yield, plant phosphorus acquisition, and mycorrhizal colonized root length, are measured. The influence of these treatments on mycorrhizal mycelium and spore production as well as on soil phosphatase (P-ase) activity was also determined. The inoculation of *G. etunicatum*, locally isolated, increased significantly the extent of P plant acquisition, spore number, length of extraradical mycelium, and P-ase activity when compared with indigenous arbuscular mycorrhizal (AM) fungi fertilized with pa-RP, at the level of 86 kg ha^{-1}. In concordance with these results, the remaining available P in the experimental soil without inoculation was depressed. A negative impact of soluble P application in *G. etunicatum* inoculated soil was noted in the P-ase activity, and also in the effectiveness of the applied inoculum in relation to P plant uptake. In soil AM inoculated and fertilized with pa-RP (86 kg ha^{-1}), the enhancement of P-ase activity was related to high mycelium development and spore formation. P plant acquisition in *G. etunicatum* inoculated plants ranged from 4.96 to 11.57 mg per pot when 86 kg ha^{-1} of pa-RP is applied compared with the same amount of soluble P. Surprisingly, adding pa-RP does not improve the amount of colonized root length. Fungal root AM colonization and AM propagules (mycelium and spores) were not depressed at higher soluble P supply, but the activity of *G. etunicatum* measured as P plant uptake was strongly affected. The inoculation with *G. etunicatum* enhances spore production, particularly at the lower soluble P and at the highest pa-RP levels. A close relationship ($r = 0.938$) between AM spores and P-ase activity was found. Only in pa-RP treatments the *G. etunicatum* inoculation reduced the external P wheat requirements. The improvement of P-ase activity by *G. etunicatum* inoculation, as a biological factor involved in P-cycling in soil, may be an important mechanism related to plant P acquisition. It is concluded that pa-RP is the best source of P, not only by increasing P uptake by interacting with *G. etunicatum*, but also by enhancing AM propagules (mycelium and spores) remaining in soil.

Keywords: P-fertilizers; Mycorrhizal propagules; Wheat; Acidic soil; *G. etunicatum*

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1. Introduction

Soil microbiology in volcanic ash-derived soils in Chile constitutes the major key factor affecting sustainability on soil fertility, in natural ecosystems not disturbed by man (Zunino and Borie, 1985). Mycorrhizal fungus participates actively to keep equilibrated relationships among non-biotic components, such as allophane and humus and the biotic ones wherein microbial forms, mainly actinomyces and fungal species, strongly interact with plant root systems at the plasmalema level. Very recently, the economy of N and S in the soil systems have been reviewed under these concepts (Aguilera et al., 2002; Borie et al., 2002). Conclusively, Chilean volcanic ash-derived soils conform a good model, to advance in the knowledge of the very complex and mutual interacting biotic and non-biotic mechanisms operating in nature to back plant nutrient availability. Among other function these mechanisms allow to keep good P-availability in such aggressive physico-chemical environment as the one induced by the allophanic clay (Borie and Zunino, 1983).

Soil sustainability is dependent on natural resource management involving soil microorganism behavior (Bethlenfalvay, 1992; Jeffries and Barea, 1994; Kennedy and Smith, 1995). In fact, particular microbial population can be managed by improving its biofertilizer activity (Barea et al., 1997). Arbuscular mycorrhizal (AM) fungi are ubiquitous present in soils, and it is very important to cooperate to define their role in plant nutrition in agricultural as well as in natural soils. Mycelium from mycorrhizal plant roots proliferate in the surrounding soil, from which they absorb nutrients of low mobility in soil (Johansen et al., 1993; Bürkert and Robson, 1994). Mycorrhizal fungi are widespread in agricultural systems (Smith and Read, 1997) and are key factors for successful low-input farming. Hence, the formation and functioning of AM symbiosis is expected to be crucial in sustainable systems (Barea and Jeffries, 1995). In agriculture, AM symbiosis is influenced by management practices, such as the amount and type of the supplied fertilizer (Bethlenfalvay, 1992; Vanlauwe et al., 2000). Soils from low-input farming systems have a greatly enhanced capacity to initiate the mycorrhizal symbiosis (Ezawa et al., 2000). The colonized root length decreases under conventional farming (Sattelmacher et al., 1991), since it is also related to general plant nutrition and nutrient availability (Douds and Schenck, 1990). Authors reported that the main cause for this effect was the amount of soil soluble P (Limonard and Ruissen, 1989; Ryan et al., 1994). Thus, the limited AM colonization found in many studies partly reflects the intensity of fertilizer input and the soluble nutrient contents, especially of P, in the soils (Johnson and Pfleger, 1992). Most studies have shown that P, among other macronutrients, presents the most incidental effect on the development of AM symbiosis. The lowering of spores viability and extraradical hyphae growth are also known to affect adversely AM symbiosis. Regarding the positive effect of increasing AM colonization on plant responses, the question raises of how fertilizer application can be managed using the appropriate doses and sources for reaching optimum nutrient—AM propagules. Furthermore, emphasis has been put on the inoculation of effective adapted mycorrhizal fungus for replacing or reinforcing the indigenous mycorrhizal population. However, the costs involved in the production of the inoculum and the large quantities required, suggest the convenience to manage indigenous populations of AM fungi (Munyanziza et al., 1997) and modeling the development of AM colonization under the effect of particular environmental conditions. The most important AM properties include: hyphal production, spore formation and AM colonization into the roots.

In the present study, we investigated the effect of two phosphate sources on the occurrence of AM propagules (spores, extraradical mycelium and mycorrhizal roots) in a pot experiment, using natural acidic soil inoculated or not with the indigenous adapted endophyte *G. etunicatum*. Wheat is a representative crop in Chilean Andisoils and it expected to express the potential capacity in AM propagules after fertilisating with different types of P-sources, under the effect of *G. etunicatum* inoculation in natural soil. The amount of AM propagules remaining in soil for subsequent crops posses ecological and practical relevance. We assumed that AM symbiosis plays an important role in plant growth and nutrition in conventionally managed low-input-systems, but the agronomic processes affecting mycorrhizal fungi under field conditions need to be explored in acidic volcanic soils.

2. Materials and methods

The soil used for the pot experiment was a Vilcún soil (Typic Dystrandepts) from Southern Chile whose main characteristics appear in Table 1. This soil shows a rather acidic pH and low available-P, in spite of its high total P content.

The experiment included eight treatments with five replications. The natural soil used was either uninoculated or inoculated with the endomycorrhizal fungus *G. etunicatum* CH 110, a native Chilean ecotype purchased from INVAM (Morgantown, WV) which had previously demonstrated to be effective in colonizing root barley, when growing in a similar soil (Borie and Rubio, 1999). Each soil was divided into four batches: two of them were fertilized with commercial triple superphosphate (soluble P) and the other two with partially acidulated-rock phosphate (pa-RP) at two rates equivalent to 17 and 86 kg P ha^{-1}.

One liter pots were filled with the soil (700 g per pot) and mycorrhizal inoculation was achieved by placing 50 g of inoculum per pot, obtained from a stock culture, consisting of mycorrhizal roots plus soil possessing fungal spores and mycelium. Saturating amount of the inoculum was placed mixed as a layer under the seedlings mixed with the soil.

Triticum aestivum L. cv. Otto was the host plant used. Wheat seeds were surface sterilized in 70 mM NaOCl for 5 min and subsequently washed with distilled water. After germination on moistened filter paper, four seedlings were planted in each pot, and 1 week after sowing, they were thinned to 2 plants per pot. Nitrogen fertilization as KNO_3 was applied as a nutrient solution at the equivalent rate of 200 kg N ha^{-1}, one-third 7 days after sowing and the remainder at the onset of flowering.

The experiment was carried out in a controlled greenhouse under a 16 h light (21 °C) and 8 h dark (15 °C) cycle, with 50% relative humidity and a photosynthetic photon flux density of 500–650 mmol m^{-2} s^{-1} for compensating photophase. During the growth periods, the plants received weekly 10 ml of Hewitt (1966) nutrient solution per pot. The pots were weighed and water losses replaced by top watering, to maintain soil moisture close to 60% field capacity during the period of plant growth. They were arranged in the greenhouse in a completely randomized design.

After a growth period of 6 months, at the milky stage of development (Zadocks 71), plants were harvested and shoots and roots were dried at 70 °C for 48 h and weighed after cooling in a dessicator. Phosphorus concentration was determined in shoot tissue using the vanado-molybdate method after an acid digestion treatment.

The grid-line intersect method (Tennant, 1975) was used to estimate root mycorrhizal colonization (stained by Phillips and Hayman, 1970) by microscopical examination and counting intersections of the grid and root according to Giovanetti and Mosse (1980).

To accomplish soil hyphae determinations, soil samples (30 g) taken from the pots at harvest were bagged and refrigerated (2–3 weeks) until the time of evaluation. Hyphal length was measured by the filtration-grid-line method (an adaptation of methods described by Kabir et al., 1997; Jakobsen et al., 1998; Bethlenfalvay et al., 1999). Soil subsamples (3 g fresh weight) were thawed and placed into flasks (250 ml) containing glycerol: 12 M HCl: distilled water (12:1:7) mixture to give a total volume of 100 ml and shaken for 30 min at 80 °C. The suspension was filtered through both 250 and 38 μm sieves. The material retained on the 38 μm sieve was resuspended in distilled water (100 ml), shaken (1 min) and allowed to stand for 30 s; a subsample of the suspension (3 ml) was transferred to a membrane filter (0.45 μm pore size, 47 mm diameter, grid-line interval 3 mm). The membrane was placed on a filter

Table 1
Selected chemical properties of soil used in this study

Available P[a]	Total P[b]	Organic P[c] (μg g^{-1})	pH H_2O	SOM[d] (%)	K (cmol + kg^{-1})	Na (cmol + kg^{-1})	Ca (cmol + kg^{-1})	Mg (cmol + kg^{-1})	Al (cmol + kg^{-1})	CEC	Al sat (%)
4.0	2540	1480	5.48	18	0.70	0.07	9.33	1.23	0.07	11.33	0.61

[a] Olsen and Sommers (1982).
[b] Dick and Tabatabai (1977).
[c] Borie and Barea (1983).
[d] Walkley and Black (1934).

holder attached to a vacuum system. After filtering, a staining solution (0.02% trypan blue in a mixture of glycerol–HCl–water) was pipetted on the membrane and allowed to stand for 10 min. The length of hyphae was quantified by using the grid-line method explained above. To express soil hyphal density on a dry soil basis, moisture content was measured after oven-drying a soil sample from each pot for 24 h at 105 °C; soil density was calculated as 0.8 g ml^{-1}.

Mycorrhizal spores were collected from soil by wet sieving and decanting according to the methodology described by Sieverding (1991).

Acid phosphatase (E.C.3.1.3.2 orthophosphoric-monoester phosphohydrolase) associated with soil was measured using an adaptation of the Tabatabai and Bremner's method (1969) and reported by Rubio et al. (1990) for other Andisols. Therefore, soil samples (100 mg) were incubated with 1 ml of 50 mM *p*-nitrophenol phosphate and 4 ml 0.1 M buffer Tris pH 5.5, for 1 h at 20 °C. At the end of the incubation period a 0.5 M $CaCl_2$ solution was added, filtered quickly receiving the filtrate over 0.5 M NaOH. Samples were centrifuged at 2500 g for 10 min. and the *p*-nitrophenol released was determined spectrophotometrically by measuring the optical density of the supernatant at 400 nm.

Soil pH was measured by glass electrode in a 1:2.5 water:soil suspension and available P using the method described by Olsen and Sommers (1982).

Data were subjected to an analysis of variance. When the main effects show significant differences among means ($P < 0.05$), they were evaluated for significance using Duncan's multiple range test.

3. Results and discussion

In general, plants grew better when partially soluble phosphate was applied as fertilizer in comparison to the soluble P source. The highest values of both shoot and root weights were obtained with plants inoculated with *G. etunicatum* (Fig. 1). Such weight increases could be explained by the pH increase (Table 2) enhancing P uptake (Table 3) with a corresponding lowering of available P levels found at the end of the experiment.

Controversy exists regarding the effect of the pH change over P solubility in soil and hence on its availability. Depending on the soil type and experimental conditions, P sorption has been demonstrated is differently affected by the increase of soil pH. Soil pH affects the charge of the P species in solution and also the charge of the adsorbing particles in volcanic soils. When pH raises, an increase in the negative charge is produced decreasing P adsorption; but, at the same time, an increase in HPO_4^{2-} concentration is produced which has a much greater affinity for reactive soil surfaces increasing consequently P adsorption. Whether a change in soil pH will increase or decrease P in soil solution sometimes depends on which effect is dominant (Whitelaw, 2000). In Chilean volcanic soils, an increase in soil pH produces a decrease in P adsorption (Mora et al., 1999). The pH increment here observed when soil is AM inoculated, is coincident with other recent findings (Borie et al., 2002), who reported a similar behavior in the same soil inoculated with the same mycorrhizal strain. The values of Olsen-P measured at the end of the experiment represents P remnant in soil solution after P root uptake. Therefore, the lowest values agree well with the highest P contents observed in shoots and roots of inoculated plants (Table 2). However, it is necessary to consider that Olsen extract is not habitually used for measuring P availability of insoluble P sources such as rock phosphate especially in the short term. In this case, the use of a partially acidulated rock phosphate could not totally reflect P availability of such P source.

Increased phosphorus uptake by AM colonized plants has been habitually selected as one of the main value for testing microsymbiont effectiveness. Thus, in the present study, this value was used to evaluating

Table 2
Soil pH and Olsen-P at harvest of wheat plants growing in a natural soil fertilized with soluble P or pa-RP at two levels (17 and 86 kg P ha^{-1}) when inoculated (I) or not (U) with *Glomus etunicatum*

Fertilizer	kg P ha^{-1}	pH		Olsen-P (mg kg^{-1})	
		U	I	U	I
Soluble P	17	5.62 d	5.46 e	31.1 c	11.0 f
	86	5.63 d	5.73 cde	32.0 c	43.2 a
pa-RP	17	5.77 bcd	6.12 a	27.7 d	25.1 d
	86	5.80 bc	5.81 bc	40.7 b	14.6 e

Values with different letters for each soil property indicate significantly different means ($P < 0.05$) by Duncan's test.

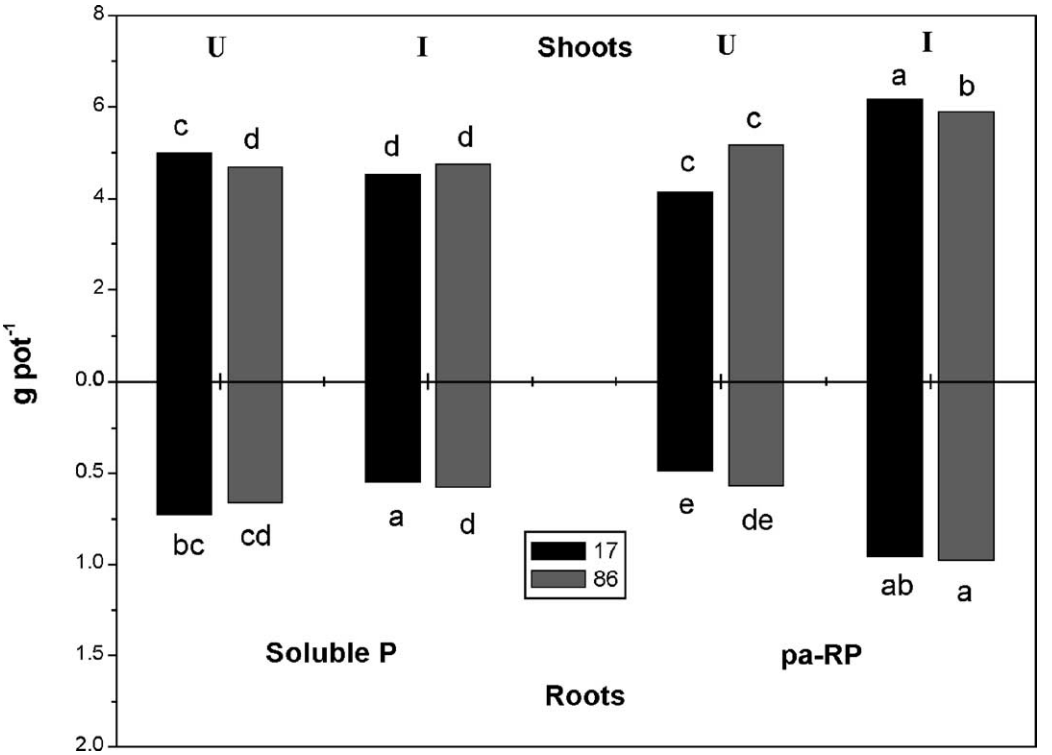

Fig. 1. Effect of mycorrhizal inoculation on shoot and root biomass (g) of wheat growing in a natural soil fertilized with soluble P or pa-RP at two levels (17 and 86 kg P ha^{-1}) and inoculated (I) or not (U) with *G. etunicatum*. Values with different letters for each mycorrhizal parameter indicate significantly different means ($P < 0.05$) by Duncan's test.

the mycorrhizal activity. High differences in P plant acquisition, according to the P sources applied, were obtained in *G. etunicatum* inoculated plants. Plant P uptake ranged from 4.96 (at 86 kg ha^{-1} soluble P) to 11.57 mg per pot (at 86 kg ha^{-1} pa-RP). The relative difference between both P sources was 133%. The positive effect of *G. etunicatum* inoculation on plant phosphorus acquisition was demonstrated only in pa-RP treatment at the highest concentration (Table 3).

Consistent P uptake improvement may also reflect the beneficial mineralizing phosphatase (P-ase) effect of *G. etunicatum* colonized roots, as well as an interaction through the introduced AMF and P-solubilizing non-symbiotic microorganisms (bacteria, fungi and

Table 3
P uptake at harvest of wheat plants growing in a natural soil fertilized with soluble P or pa-RP at two levels (17 and 86 kg P ha^{-1}) when inoculated (I) or not (U) with *G. etunicatum*

Fertilizer	kg P ha^{-1}	Shoot P concentration (mg g^{-1})		Shoot P content (mg per pot)	
		U	I	U	I
Soluble P	17	1.69 a	1.77 a	8.18 bc	8.67 abc
	86	1.29 b	0.79 c	6.17 e	4.96 f
pa-RP	17	1.88 a	1.53 b	8.12 bcd	9.47 abc
	86	1.41 b	1.97 a	7.60 de	11.57 a

Values with different letters for each plant parameter indicate significantly different means ($P < 0.05$) by Duncan's test.

Table 4
Root colonization and mycorrhizal propagules left in the soil at harvest of wheat plants growing in a natural soil fertilized with soluble P or pa-PR at two levels (17 and 86 kg P ha^{-1}) when inoculated (I) or not (U) with *G etunicatum*

Fertilizer	kg P ha^{-1}	Root colonization (%)		Colonized root length (m per pot)		AM Spores (no. 100 gds^{-1})		Hyphal density (m ml^{-1})	
		U	I	U	I	U	I	U	I
Soluble P	17	50.3 b	67.0 a	27.9 b	23.2 b	185 ef	1825 a	13.8 bc	14.3 bc
	86	45.3 b	40.5 bc	32.6 a	18.7 c	146 f	283 de	11.4 c	16.6 b
pa-PR	17	50.8 b	43.3 b	23.9 b	27.3 b	331 d	633 c	11.9 c	12.2 c
	86	40.7 bc	36.6 c	22.1 b	21.5 b	254 e	1295 b	14.1 bc	21.0 a

Values with different letters for each mycorrhizal parameter indicate significantly different means ($P < 0.05$) by Duncan's test.

actinomycetes). They have been reported to be able to bring insoluble phosphates in soil into soluble forms by producing organic acids (Asea et al., 1988). Results reported by Young (1990) demonstrated that the effectiveness of P-solubilizing bacteria in acidic soils was greater than in a calcareous soil. In this study, P plant acquisition was enhanced by manipulating the mycorrhizal status of the rhizosphere with AMF inoculation plus natural RP fertilizer application, particularly in doses higher than 17 kg ha^{-1}. This nutritional response could corroborate previous findings reported by Borie et al. (1983) concerning the effectiveness of some native fungal strains, isolated from Chilean Andisols, in solubilizing insoluble phosphates including Ca, Fe and Al phosphates and phytates, and also commercial rock phosphate. It is important to remark that Fe and Al inositol phosphates are the main organic P forms found in these soils (Borie et al., 1989). Some studies have confirmed that utilization of organic P is enhanced by AM (Joner and Jakobsen, 1994). The effect of *G. etunicatum* colonization on P uptake seems to be caused by an enhancement in root surface area by increased AM mycelium and also phosphatase activity (Tarafdar and Marschner, 1994; Li et al., 1997). Both activities were widely increased in the most efficient treatment in P uptake (Tables 3 and 4, Fig. 2).

In this work, values of mycorrhizal mycelium and also P-ase activity increase in *G. etunicatum* colonized plants treated with 86 kg ha^{-1} pa-RP. Given the case, that organic P seems to be the sink for P after decades of P-fertilization management in Chilean volcanic soils (Borie and Zunino, 1983), these results could explain the relative contribution of soil organic sources of P to the mycorrhizal plant P nutrition in this experimental soil (with 58% of total as organic P, Table 1) when extraradical mycelium was well developed (Table 4, Fig. 2).

The use of soluble P affected more intensively the P uptake activity of the *G. etunicatum* colonized plants (Table 3) than its colonizing ability and propagules production (Table 4). The length of root AM colonized by *G. etunicatum*, in soluble P fertilized soil was not effective for P plant acquisition at the lower dose (17 kg P ha^{-1}), and was detrimental at the higher one (86 kg P ha^{-1}).

The results of this investigation show that under natural conditions, the addition of pa-RP, at whatever concentration used, resulted equal (non-inoculated soil) or more efficient (inoculated with *G. etunicatum*) than the highest soluble P applied in wheat P acquisition (Table 3). The most compatible interaction between pa-RP (at 86 kg P ha^{-1}) and *G. etunicatum* in natural soil increased shoot phosphorus content by 52 or 40%, when expressed as P concentration, over non-inoculated plants; this effect could be associated with an increased extraradical mycelium development and P-ase activity in soil. According to these results, the assessment of hyphal development and length of mycorrhizal root is more indicative than spores production to evaluate the functionality of the AM symbiosis.

Phosphorus is frequently the most limiting element for crop production in nearly all ecosystems, but the application of heavy doses of P fertilizer reduces the population and physiological activity of AM fungi (Krishna and Bagyaraj, 1982; Bagyaraj, 1990; Guillemin et al., 1995). In agreement with this statement, Miller and McGonigle (1992) reported that a reduction in mycorrhizal colonization resulted in a decrease of phosphorus absorption in AM colonized

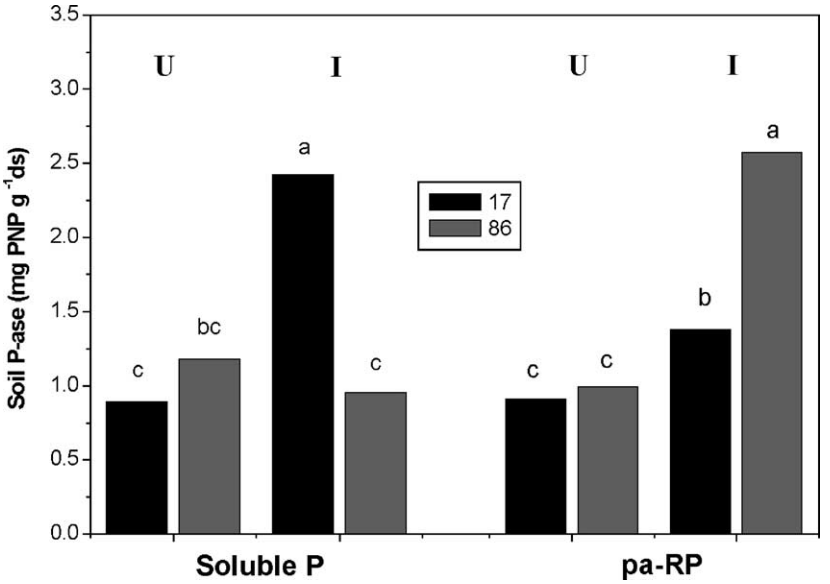

Fig. 2. Effect of mycorrhizal inoculation on soil phosphatase after wheat growing in a natural soil fertilized with soluble P or pa-RP at two levels (17 and 86 kg P ha^{-1}) and inoculated (I) or not (U) with *G. etunicatum*. Values with different letters for each mycorrhizal parameter indicate significantly different means ($P < 0.05$) by Duncan's test.

plants; but in this study, as Table 4 shows, mycorrhizal colonization of roots was not eliminated at the higher soluble P application, although it was detrimental for P plant acquisition.

The effectiveness of pa-RP on P plant acquisition is more related to extraradical values (P-ase activity and mycelium) than to mycorrhizal root length (Table 4, Fig. 2). Extraradical hyphae proliferation having the dual P-mineralizing and P uptake abilities as well as successful interaction with natural RP solubilizing microorganisms can explain such effect. Here, results suggest (as in previous studies from Tarafdar and Marschner, 1995; Song et al., 2000; Feng et al., 2002) that *G. etunicatum* colonized roots were able to produce acid phosphatase that is released into the soil and also that P from organic compounds can become available to plants, after hydrolysis by phosphatase enzymes.

Mycorrhizal colonization developed by *G. etunicatum* in pa-RP treatment was more efficient on P acquisition than the rest of the treatments applied (Table 3) independent of the mycorrhizal root length (Table 4). Nevertheless, the highest amount of extraradical mycelium produced at this pa-RP amount correlates well with the maximum P-ase activity and with mycorrhizal response on plant P acquisition.

Mäder et al. (2000) observed that soils from the low-input farming system had a greatly enhanced capacity to initiate AM symbiosis. In general, P availability from rock phosphate is often too low to demonstrate an immediate impact on cereal production. However, in this work it is demonstrated that AM inoculation and the addition of pa-RP to soil increased rapidly the soil available P-pool, improving the soil fertility status.

Extraradical mycelium production in natural soil was not changed by P amendments (Table 4). However, the inoculation with *G. etunicatum* increased the mycelial development, particularly with 86 kg ha^{-1} of pa-RP. This indicates that some AM fungi are more sensitive to P fertilization than others (Miranda de and Harris, 1994). Similarly, contrasting responses were determined by Douds and Schenck (1990) for sporulation outside the roots.

The *G. etunicatum* inoculation highly enhanced the number of AM spores in the soil at any level and P-source (Table 4). Spore increments were the highest at the greater level of pa-RP (86 kg ha^{-1}) and also at

the lower soluble P doses (17 kg P ha^{-1}). Comparing these values with the results from Table 2, we can see that the lowest amount of available P in the remaining soil after yield was determined in both P-treatments. A direct negative correlation between spores number and available P in soil seems to be clear ($r = -0.872$). Results by Douds and Schenck (1990) reported that production of soilborne spores by AM fungi was consistently increased when P was lacking in the soil nutrient solution. However, Ezawa et al. (2000) reported that P accumulation did not reduce the density of the spores of the dominant species. According to Muthukumar and Udaiyan (2000), the host may directly influence and control AM fungal sporulation, by regulating AM intraradical fungal structures (vesicles and arbuscules). Nevertheless, carbohydrate concentration in the root has to reach a low critical value for sporulation starting, and spore numbers in the soil were correlated with P and K in plant, but not in soil (Muthukumar and Udaiyan, 2000). In fact, sporulation of AM fungi can be enhanced by manipulation of nutrient regimes in the pot culture, especially N:P ratios (Douds and Schenck, 1990); consequently, deeper studies are required to understand these effects under natural conditions. Authors, such as Mosse (1986), also reported no clear relationship between incidence of AM propagules and nutrient application. Nevertheless, AMF from different genera respond differently to management by agricultural practices (Boddington and Dodds, 2000).

Indigenous mycorrhizal fungi normally differ in their capacity to form propagules (Abbott and Gazay, 1994). The relationship between root colonization and propagule formation for different fungi in field environment is not well understood (Abbott and Gazay, 1994). Nevertheless, in this natural soil, having the indigenous AM populations, the application of pa-RP increase effectively the number of AM spores. Moreover, *G. etunicatum* inoculation also increased spore numbers, but spore populations were not directly related to the rate of mycorrhizal formation which agrees with Abbott and Robson (1982) report. Thus, we have the possibility to improve the AM potential of natural soils by increasing the number of spores through pa-RP fertilization practices together with *G. etunicatum* inoculation.

Mäder et al. (2000) recommended that for enhancing AM symbiosis practices, factors favoring optimal soil properties, such as management of nutrient content for the development and activity of AM symbiosis should be chosen.

From an ecological point of view, mycorrhizal root length, extraradical mycelium and spores represent the main AM propagules. Mycelium density as AM propagule was considered important to understand the influence of soil management strategies such as fertilization sources and doses (McGonigle et al., 1990). Extraradical hyphae are reported to be the main source of inoculum in soil (Sylvia, 1992; Requena et al., 1996). This aspect is important as long as it could remains active from one crop to the succeeding one in a crop rotation period.

In the present study, nutrient application not only affected mycorrhizal activity but also infective propagules, such as number of spores, mycorrhizal root length or mycelium production; nevertheless, not all these values seem to be responsible for the mycorrhizal differences found in plant P uptake. Sequential harvesting will allow a more accurate evaluation about propagules development and the relevance on plant nutrition under particular fertilizer conditions.

Changes in soil conditions will modify the dominance of any particular fungi during the AM colonization in field soil, but the extent to which the most suitable fungi would dominate as soil conditions change, is not known and requires biodiversity studies. These soils must be successfully managed to enhance the fungal propagules and the ability of efficient fungi to colonize a greater proportion of the root system.

Soil phosphatase activity (Fig. 2), as an enzymatic value able for mineralizing organic P is related to the biological soil P cycling and it was enhanced by *G. etunicatum* inoculation, in nearly all the cases. Under the applied P treatments, P-ase did not decrease with soluble P in natural soil in inoculated plants and rose when the highest pa-RP dose was applied, but it decreased at similar level of soluble P. This mineralizing activity may also account for the P plant uptake tested in this soil, where organic P account for more than half of total P (Table 1).

Joner and Johansen (2000) determined that up to 70% of the measured phosphatase activity was associated with the hyphal wall and the rest with internal structures. The same authors have also reported differences among AM isolates in relation to P-ase activity. The soil with native fungi showed a lower

P-ase activity, compared with the soil inoculated with *G. etunicatum*.

The negative correlation between P-ase and Olsen-P in soil ($r = -0.8674$) indicates that as much as soluble P was in the growing medium less expressed was P-ase activity.

To increase P plant nutrition, nutrients have to be either supplied as fertilizer, or conditions for mycorrhizal formation have to be favored. A compromise between both components (fertilizer and AM inoculum) acting in a compatible way is suggested. In this study, the applications of the slow released phosphate (pa-RP) fertilizer especially at higher rate have beneficial effect on the activity of AM symbiosis. As results show, there are stimulating effects of pa-RP and AM inoculation on AM propagules, root development and P mobilized using a local AM isolate strain under natural conditions. Moreover, such natural source of P influenced the amount of AM propagules remaining in soil for a subsequent crop.

In summary, bearing in mind our findings in relation to benefits obtained with partially soluble phosphate in terms of plant growth together with AM propagules released and left in the soil for subsequent crop, it is important to conclude that farmers must intensify the use of insoluble P sources instead soluble ones. In the agriculture of Southern Chile rock phosphate be alone or partially acidulated are applied almost exclusively in prairies but this study suggests its use in annual crops as cereals are.

More studies are needed to fully understand the unusual and unique biological and chemical mechanism operating in Chilean volcanic soils. Undoubtedly, allophane physico-chemical activity should be incorporated as a condition that is permanently cooperating to keep ecologically equilibrated plant nutrition processes in these soils. As far as we are able to perform this, we will develop the best agronomic management to attain the full yield capacity by volcanic soil systems.

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