Ecology / Ecología

Effects of organic acid application and ectomycorrhizal symbiosis on growth and morphological parameters of _Pinus pseudostrobus_

Mariela Gómez-Romero[1,2], Roberto Lindig-Cisneros[3], Ana Laura Cruzado-Vargas[3] and Ek del-Val[3*]

[1] Escuela Nacional de Estudios Superiores Unidad Morelia. Universidad Nacional Autónoma de México. Morelia, Michoacán, México.
[2] CONAHCYT- Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, México.
[3] Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Morelia, Michoacán, México.
*Corresponding author: ekdelval@iies.unam.mx

Abstract

Background: Many landscapes, both natural and anthropogenic, are dominated by degraded soils that have low phosphorus availability due to low overall phosphorus concentration or to phosphorus sequestration by iron-rich minerals.

Questions and / or Hypotheses: Does the application of low molecular weight organic acids improve phosphorus availability and plant growth in phosphorus-poor soils, and is this effect modulated by ectomycorrhizal fungi?

Studied species / data description /Mathematical model: _Pinus pseudostrobus_ and its ectomycorrhiza _Pisolithus arhizus,_ in addition to six sodium salts of organic acids.

Study site and dates: The experiment was carried out in a shade house (35 % shade) in Morelia, Michoacán in 2015.

Methods: We conducted experiments with _Pinus pseudostrobus_ and its ectomycorrhiza _Pisolithus arhizus_ in addition to six sodium salts of organic acids. Sodium salts of citrate, oxalate, acetate, tartrate, succinate and malate were added to the soil at 0, 4, 8, 16, 32 and 64 micromolar concentrations.

Results: The salts of the organic acids—particularly tartrate and malate-solubilized phosphorus and improved plant growth after 12 months in the absence of _P. arhizus_. When plants were inoculated with _P. arhizus_, the effect of most organic acids was either detrimental or non-significant. However, citrate, tartrate and succinate improved biomass and morphological parameters.

Conclusions: These results suggest that adding appropriate organic acids to heavily degraded soils can aid _P. pseudostrobus_ establishment and its benefits are higher than the association with mycorrhiza for young plants during the initial stages of fungal colonization.

Key words: mycorrhizal, phosphorus, pine, soil restoration.

Resumen

Antecedentes: Actualmente muchos paisajes están dominados por suelos degradados con baja disponibildiad de fósforo, debido a una baja concentración en el suelo o al secuestro del fósforo por minerales ricos en hierro.

Preguntas: ¿Los ácidos orgánicos pueden aumentar la disponibilidad de fósforo y el crecimiento de plantas en suelos bajos en fósforo? De ser así, ¿estos efectos están modulados por hongos ectomicorrícicos?

Especies de estudio: _Pinus pseudostrobus_ y su hongo ectomicorrícico _Pisolithus arhizus._

Sitio y años de estudio: El experimento se realizó en una casa de sombra (35 % de sombra) en Morelia Michoacán en 2015.

Métodos: Realizamos dos experimentos con _Pinus pseudostrobus_ y su hongo ectomicorrícico _Pisolithus arhizus_ adicionando seis sales de ácidos orgánicos. Las sales de citrato de sodio, oxalato de sodio, acetato de sodio, tartrato de sodio, succinato de sodio y malato de sodio se añadieron en las siguientes concentraciones 0, 4, 8, 16, 32 y 64 micromolar.

Resultados: Las sales orgánicas, particularmente tartrato y malato, solubilizaron el fósforo e incrementaron el crecimiento de las plantas después de 12 meses en ausencia de _P. arhizus_. En los pinos inoculados con _P. arhizus_, solamente el citrato, tartrato y succionato aumentaron la biomasa y algunos caracteres morfológicos.

Conclusiones: Nuestros resultados sugieren que añadir los ácidos orgánicos apropiados a suelos severamente degradados, pueden ayudar en el establecimiento de los pinos y sus efectos positivos fueron mayores que la asociación con micorrizas para plantas durante las etapas iniciales de colonización por los hongos.

Palabras clave: fósforo, micorrizas, pinos, restauración de suelos.

Phosphorus is the limiting macronutrient in most continental soils, mainly because of its mineral origin and poor diffusion in soils (Johan *et al.* 2021). Unlike nitrogen, the processes that regulate the phosphorus cycle are mostly abiotic. Some of the key factors that limit phosphorus availability to plants are those that determine phosphorus adsorption and desorption from the mineral matrix, particularly in the presence of iron and aluminum compounds that limit the presence of this element in soluble forms (Hekstra 1996). Phosphorus deficiency can also be exacerbated by some management practices. For example, nitrogen fertilizers can increase soil acidity, which favors insoluble forms of phosphorus (García-Oliva 2005) and inhibits symbiotic relationships (Grant *et al.* 2005). At the same time, mycorrhizal fungi can establish symbiotic relationships with many different plant species, van der Heijden *et al.* (2015) estimate that there are close to 50,000 fungal species that form mycorrhizal associations with up to 250,000 plant species. Several studies have shown that arbuscular mycorrhiza fungi contribute up to 90 % of plant P uptake (van der Heijden *et al.* 2015, Strullu-Derrien *et al.* 2018). Additionally, mycorrhizae improve the formation and stabilization of soil aggregates (Tisdall 1994, Bearden 2001). This in turn leads to major changes in plants' physiology and responses to the environment (Ferrera Cerrato & Alarcón 2001) and increases soil bacterial diversity (Álvarez 2009). However, there is apparently some initial cost to plants of establishing these symbiotic relationships, as plants have been repeatedly shown to exhibit early growth depression during the initial stages of colonization by mycorrhizae (Bethlenfalvay 1982, Li *et al.* 2005, Choi *et al.* 2005, Smith & Read 2008, Barroetaveña *et al.* 2012, Gómez-Romero *et al.* 2013). Plants found in soils with low phosphorus content may have a mycorrhizal dependency. Even though has been documented with arbuscular mycorrhizal fungi, that colonization will be variable, depending on phosphorus concentrations in the soil. In this sense, the application of phosphorus can reduce mycorrhizal colonization. In general, plant species with a lower ability to take up phosphorus are recognized as dependent on mycorrhiza. This is why when phosphorus levels in the soil increase, mycorrhizae may not benefit (Graham *et al.* 1991, Janos 2007).

Organic acids are part of the soluble fraction of the rhizosphere and influence the availability of nutrients, including phosphorus (Adeleke *et al.* 2017, Baltazar *et al.* 2021). Plant roots can exude some organic compounds that favor the solubilization of phosphorus from iron or aluminum minerals, thus increasing phosphorus availability (Adeleke *et al.* 2017). Plants can release organic acids that can bind to cations and in the process release phosphorus into the soil without ectomycorrhizal symbiosis (Ae *et al*. 1990, 1991). However, when ectomycorrhizal symbionts are present, their mycelium increases plants' phosphorus uptake considerably (Chuyong *et al.* 2000) through several mechanisms: 1) an increase in the absorption surface, which allows more efficient exploration of the soil volume; 2) increased surface area of contact with soil particles; 3) formation of polyphosphates; and 4) release of organic acids and phosphatases that solubilize phosphorus by binding to cations in the soil (Marschner & Dell 1994, Bücking 2004). After a thorough review of the knowledge on mycorrhizal ecology and evolution, van der Heijden *et al.* (2015) conclude that paleontological and phylogenetic evidence indicate that the mutualistic relationship is very old (ca. 450 million-yr-old), and that it probably allowed plant transition from water to land.

The mutualism between gymnosperms and ectomycorrhizal fungi have been thoroughly studied, and many fungal genera are commonly used to improve gymnosperm growth (Pera & Parladé 2005). *Pisolithus arhizus* (previously known as *Pisolithus tinctorius*) is a gasteroid, globose, ectomycorrhiza that has been used with different trees and shrubs to increase their overall growth and root length and volume, as well as phosphorus nutrition, especially in soils with phosphorus deficiency (Gómez-Romero *et al*. 2015, Becerril-Navarrete *et al*. 2022). The inoculation with *P. arhizus* has been reported with forest plants from the five continents (Cairney & Chambers 1997) including Pinaceae family (Pérez-Moreno & Read 2004). *Pisolithus arhizus* is a cosmopolitan species, distributed in all regions of the country. Widely cited in the states of north, center and south (Chihuahua, Coahuila, State of Mexico, Hidalgo, Jalisco, Nuevo León, Oaxaca, Sonora and Veracruz; Bautista-Hernández *et al.* 2018). On the other hand, this species of ectomycorrhiza provides resistance to water stress in *Pinus pseudostobus*. The conditions at the substrate collection site are a highly degraded site with serious drought problems, so *P. arhizus* is of vital importance at the site (Gómez-Romero *et al.* 2015). The objective of the present study was therefore to explore the effect of different organic acids, in non-inoculated and inoculated (with *Pisolithus arhuizus*) *Pinus pseudostrobus* plants under controlled conditions.

We hypothesized that organic acids would improve plant performance for both non-inoculated and inoculated plants, and that adding organic acids would reduce the initial cost of the ectomycorrhizal symbiosis in small plants.

Materials and methods

Locally collected seeds (Nuevo San Juan Parangaricutiro, Michoacán) of *Pinus pseudostrobus* were cold stratified for 15 days at 4 °C in Petri dishes lined with moistened Whatman filter paper (No. 1), after being superficially sterilized with 20 % sodium hypochlorite (NaClO 1:5 H_2O). The seeds were then placed in a growth chamber at 25 °C with a 12:12 hour photoperiod until they germinated. The germinated seeds were planted in plastic containers with 50 cm³ of a substrate composed of a 1:1 mixture of commercial peat moss and agrolite and kept in the growth chamber for another two weeks. The substrate was previously sterilized in an autoclave at 100 °C for two periods of 20 minutes. The seedlings were then transplanted into 375 cm³/ 600 g containers with sterilized degraded acrisol-like substrate. The substrate used comes from a deforested site that is mostly vegetation devoid, with the presence of numerous gullies. The place is severely eroded, with presence of acrisols. Soil analyzes indicate that the phosphorus content is extremely poor (Table 1).

Table 1. Description of the physical and chemical characteristics of the substrate used in the experiments.

Physical analysis	Clay-silt-sand	71.80 -16 -12.20
	Clase textural	Heavy clay
	Apparent density	1.20
	Field capacity	43.16
	Permanent wilting point	23.45
	Usable moisture	19.54
	Saturation humidity	56
	Porosity (%)	45.6
Chemical analysis	pH in water	5.4 Moderately acidic
	pHCaCl$_2$	4.9 Acid
	C.E. milimohos	0.06 Deficient in salts
	% O.M.	0.83 Poor
	Organic Nitrogen kg/ha	20.88 Poor
	Ammoniacal nitrogen (ppm)	8.4 Very poor
	Phosphorus kg/ha	Traces Extremely poor
	Potassium kg/ha	36.88 Very poor
	Calcium kg/ha	1148 Very poor
	Magnesium kg/ha	133 Medium
	C.E.C.	28.40

In our first series of experiments, we tested the effect of organic acids salts on the performance of *P. pseudostrobus* plants, with and without inoculation with *Pisolithus arhizus*. Each experiment consisted of six replicates of each of six dilutions (0, 4, 8, 16, 32 and 64 micromolar) of the sodium salts of citrate, oxalate, acetate, tartrate, succinate, and malate, we used these concentrations as that is has been reported that across a broad range of ecosystems, the concentration of organic acids in soil range from ranges from 0 to 50 μM (Adeleke *et al*. 2017). To provide macro- and micronutrients, we prepared a hydroponic solution consisting of the aqueous solution of the following salts: KNO_3 (202 mg/l), $Ca(NO_3)_2$ (236 mg/l), $MgSO_4$ (493 mg/l), NH_4NO_3 (80 mg/l), H_3BO_3 (2.8 mg/l), $MnSO_4$ (2.07 mg/l),

$ZnSO_4$ (0.22 mg/l), $CuSO_4$ (0.051mg/l) and $(NH_4)_6MO_7O_{24}$ (0.09 mg/l). (Modified from Salisbury & Ross 1994). The source of phosphorus was *Iron (III) phosphate dihydrate,* an insoluble form of this element; 1 g was mixed into the substrate of each plant. The organic acid salts were added twice per week and the hydroponic solution three times per week. Lixiviates were collected and analyzed using the molybdenum blue method (Frank *et al*. 1998). For the inoculation of experimental plants with the mycorrhiza, each plant was treated with *ca.* 500,000 spores of *Pisolithus arhizus* obtained from the company Biosyneterra Solutions Inc. (L'Assomption, Quebec, Canada). The spores were added to 0.5 g of micronized peat as a vehicle, which was applied directly to a portion of root that had been exposed by moving the substrate. Height, stem diameter and canopy cover were measured every month for six months. During the seventh month, many of the plants were destroyed by insect herbivores, therefore only six-month data was analyzed as early performance response of the plants. A second set of experiments was conducted as described above, but using only non-inoculated plants and quantifying height, stem diameter, canopy cover, shoot biomass and root biomass for a longer period: 12 months.

Experiments were performed in a shade house (35 % shade) whose conditions were very similar to those in the habitat where seeds were collected. The temperature in the shade house ranged between 13 and 29 °C depending on the month, humidity was not controlled since the shade house is open, but all plants were subject to the same irrigation regimes during both experiments. For statistical analyses of the first experiment, we used ANOVA to test effects of presence-absence of mycorrhiza and organic acid salt identity. For both experiments we used linear models to test the effects of the organic acid salts concentration on plant response variables. For all tests we verified the fulfillment of the assumptions of the procedures. We included both linear and polynomial fits and retained quadratic polynomial variables whenever this improved the overall fit of the model (*i.e.*, increased the model's adjusted R^2 value). All statistical analyses were done in R (R Core Team 2020).

Results

The results of the first set of trials after 6 months showed significant differences on plant performance for the presence or absence of mycorrhiza, for: height ($F_{(1,636)}$ = 89.7; $P < 0.0001$), stem diameter ($F_{(1,635)}$ = 105.9; $P < 0.0001$) and canopy cover ($F_{(1,635)}$ = 4.8; $P = 0.03$). For height, plants without mycorrhiza were taller than plants with mycorrhiza (7.06 ± 1.7 and 5.9 ± 1.3 cm, respectively), stem diameter was larger for plants without mycorrhiza than for plants with it (0.9 ± 0.2 and 1.2 ± 0.4 mm, respectively), and for canopy cover plants had greater cover without mycorrhiza than for plants with it (22.6 ± 8.3 and 21.0 ± 9.3 mm², respectively). The effect of organic acid salt identity was significant for stem diameter ($F_{(5,635)}$ = 27.1; $P < 0.001$), and canopy cover ($F_{(5,635)}$ = 4.3; $P < 0.001$).

When considering the effect of each organic salt concentration in the first experiment, for plants without mycorrhizal inoculation (Table 2), increasing concentration of organic acid salts had non-significant or negative effect on response variables. This was the case of citrate (for height, stem diameter, and canopy cover), of tartrate (for height and stem diameter) and for oxalate and succinate (both for canopy cover). In plants inoculated with mycorrhizae, there were negative effects of acid concentration of citrate on stem diameter, tartrate on stem diameter and malate on canopy cover.

For the second set of trials, in non-inoculated plants, the results after 12 months were different. In this case, when organic acids showed a significant effect, those effects were positive (Figure 1). Stem diameter increased as the concentration of citrate, oxalate, succinate, or tartrate salts were increased, the best fit being linear for all (Figure 1A-D). All organic acid salts significantly increased canopy cover as their concentration increased; for acetate and tartrate the best fit was linear, while for the rest of the salts the best fit was quadratic polynomial (Figure 1E-J). Interestingly, when the best fit was a curve, the inflection point of the curve was around the concentration of 40 micromolar. Citrate, oxalate, and succinate salts had a positive effect on shoot biomass, in all cases being the best fit polynomial (Figure 1K-M), with an inflection point close to the concentration of 40 micromolar. Oxalate, succinate and tartrate salts had a positive effect on root biomass, and the best fit was polynomial in all cases (Figure 1N-P). For oxalate and tartrate salts, the inflection point is near 40 micromolar, though this was not the case for succinate.

Table 2. Effect of organic acid addition on different *Pinus pseudostrobus* performance variables in experiments with and without the ecto-mycorrhiza *Pisolithus arhuizus*. Experiment 1 had only un-inoculated plants. Only significant effects are shown. Significance codes: 0 '***', 0.001 '**', 0.01 '*' specifying when the relationship was significantly positive (+), negative (-) or polynomial (polynomial). N/E: not evaluated.

Organic Acid	Response Variable	Experiment 1		Experiment 2
		Without mycorrhiza	With mycorrhiza	Without mycorrhiza
Sodium citrate	Height	** (-) $R^2 = 0.16$		
Sodium tartrate	Height	* (-) $R^2 = 0.12$		
Sodium citrate	Stem diameter	** (-) $R^2 = 0.15$	* (-) $R^2 = 0.08$	* (+) $R^2 = 0.12$
Sodium oxalate	Stem diameter			** (+) $R^2 = 0.25$
Sodium succinate	Stem diameter			*** (+) $R^2 = 0.28$
Sodium tartrate	Stem diameter	* (-) $R^2 = 0.08$		* (+) $R^2 = 0.13$
Sodium acetate	Canopy cover			* (+) $R^2 = 0.27$
Sodium citrate	Canopy cover	*** (-) $R^2 = 0.42$	* (-) $R^2 = 0.12$	** (polynomial) $R^2 = 0.29$
Sodium malate	Canopy cover		*** (-) $R^2 = 0.19$	** (polynomial) $R^2 = 0.44$
Sodium oxalate	Canopy cover	** (-) $R^2 = 0.14$		** (polynomial) $R^2 = 0.26$
Sodium succinate	Canopy cover	* (-) $R^2 = 0.07$		** (polynomial) $R^2 = 0.36$
Sodium tartrate	Canopy cover			** (+) $R^2 = 0.15$
Sodium citrate	Shoot biomass	N/E	N/E	*** (polynomial) $R^2 = 0.45$
Sodium succinate	Shoot biomass	N/E	N/E	* (polynomial) $R^2 = 0.24$
Sodium tartrate	Shoot biomass	N/E	N/E	** (polynomial) $R^2 = 0.30$
Sodium oxalate	Root biomass	N/E	N/E	** (polynomial) $R^2 = 0.35$
Sodium succinate	Root biomass	N/E	N/E	*** (polynomial) $R^2 = 0.89$
Sodium tartrate	Root biomass	N/E	N/E	** (polynomial) $R^2 = 0.18$
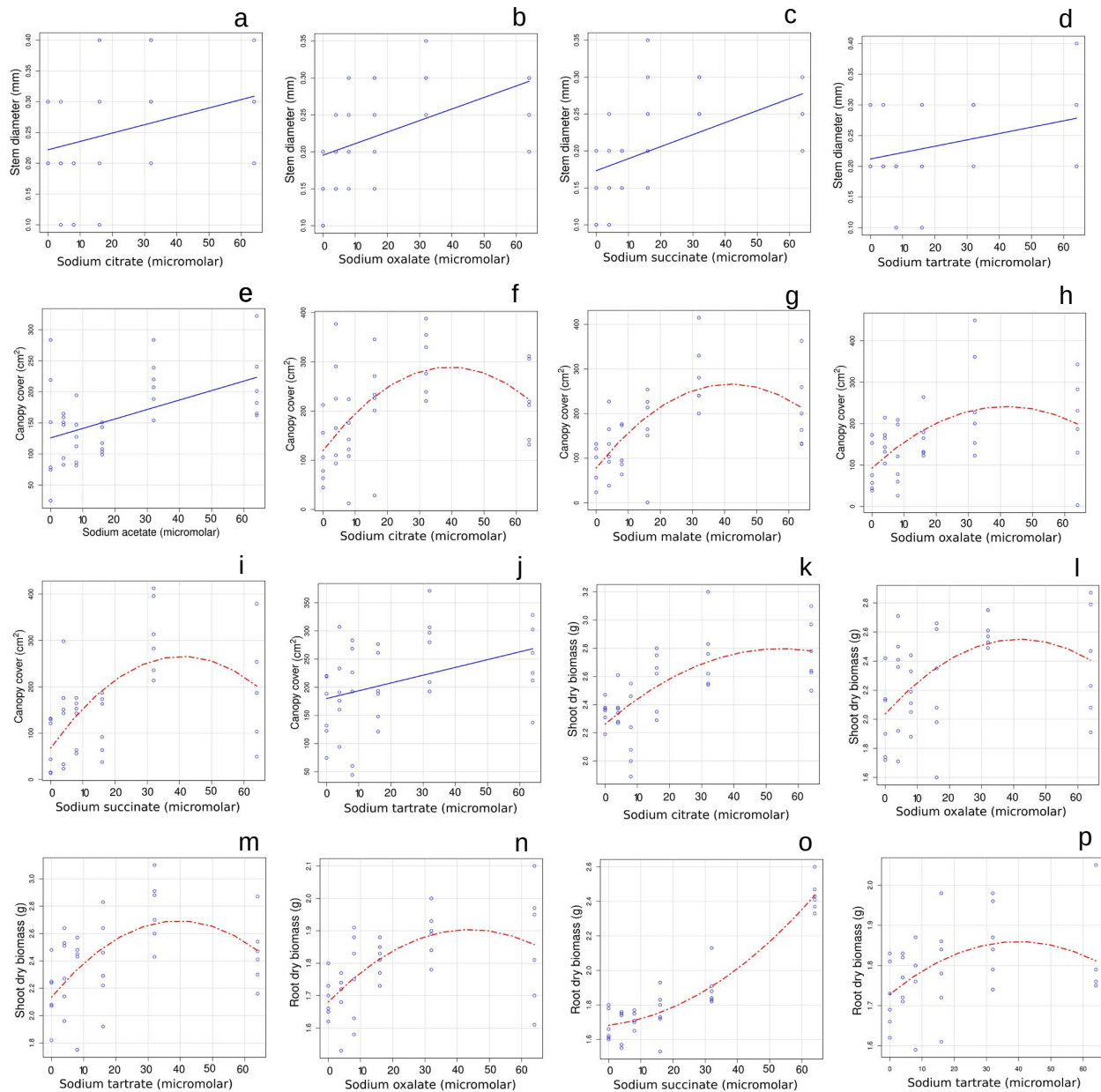
Figure 1. Four plant performance variables for *Pinus pseudostrobus* to increasing concentrations (0, 4, 8, 16, 32 and 64 micromolar) of organic acid sodium salts after 12 months in plants not inoculated with mycorrhizae (Experiment 2). Effects on Stem diameter: A) citrate, B) oxalate, C) succinate, D) tartrate; on Canopy cover E) acetate, F) citrate, G) malate, H) oxalate, I) succinate, J) tartrate; on Shoot dry biomass: K) citrate, L) oxalate, M) tartrate; on Root dry biomass: N) oxalate, O) succinate and P) tartrate. Blue lines denote significant linear relations while red lines denote significant quadratic relations. We are only showing the variables and treatments that showed significant effects.

Discussion

The process of phosphorus absorption by roots is directly related to aspects of root morphology, which determine the efficiency with which the root system explores the soil. It also depends on chemical processes involving proton and low-molecular-weight organic acid excretion (Junk *et al.* 1993). Our results indicate that when added directly to the growing medium, organic acids have positive effects on plant performance, but the magnitude and specific growth

variables promoted differ. Several studies have shown that plants release organic acids when available phosphorus concentrations in the soil are low (Peñaloza *et al.* 2000, Tian 2004). For example, there are a handful of studies of the organic acid root exudates of pines (Wang *et al.* 2007, Johansson *et al*. 2008, 2009, Meier *et al*. 2013), and for *Pinus radiata*, the profile of organic acid exudates has been fully characterized (Shi *et al*. 2011). That species produces mainly: formate, acetate and malate, and to a slightly lesser degree: lactate, shikimate, succinate and tartrate (Shi *et al*. 2011). It has been documented that the application of citric acid, can improve growth, biomass accumulation and chlorophyll content (Chen *et al.* 2020). On the other hand, malic acid increases plant productivity, showing greater growth by alleviating stress from the presence of metals (Zhang *et al*. 2020). This acid increases biomass in *Salix variegata* (Zhang *et al*. 2020) and in *Oriza sativa* (Sebastian & Prasad 2018). Oxalic acid also increases plant productivity (Guo *et al*. 2019) and can counteract stress effects, for example in *Cicer arietinum* (Sakouhi *et al*. 2022) or in *Sedum alfedii* (Liang *et al*. 2021).

Interestingly, in the literature oxalate and citrate are frequently reported as exudates by bacteria and mycorrhiza (Banik & Dey 1982, Gyaneshwar *et al.* 1998, Ahonen-Jonnarth *et al.* 2000, Sheng & He 2006, Eldhuset *et al.* 2007, Vyas & Gulati 2009, Sheng *et al.* 2011), in our study these organic acids showed a significant effect on four and three response variables (the only variable for which citrate had no effect was root dry biomass). A study by Ohno & Kubicki (2020) on phosphorus availability in the presence of organic acids, including four of the organic acids examined in the current study (oxalic, citric, succinic, and malic acids), showed that phosphorus adsorption to FeOOH was the lowest in the presence of oxalic and citric acids; malic acid also reduced adsorption, but succinic acid did not. These adsorption results are consistent with ours except for succinate, which had a significant effect on 3 response variables suggesting that other mechanisms might be at play, including the presence of exudates by the plants themselves or the pH of the growing medium, that influences the deprotonation of the carboxyl group (Ohno & Kubicki 2020). Some studies have shown that succinate is a key molecule in promoting plant growth (Iyer *et al.* 2017). Also, tartrate has been shown to play a role in phosphorus solubilization of iron-bound phosphates (Shen *et al.* 2002) and that malate plays a role in increasing phosphorus assimilation in species such as *Lupinus albus* (Johnson *et al.* 1996). For our results, it is interesting that the best fit for increase in plant performance under the different organic acid addition in seven cases was polynomial, with an inflection point of the curve close to 40 micromolar, suggesting that high concentrations of the organic acids may no longer promote plant performance after those concentrations, those concentrations may be detrimental.

Mycorrhizal inoculation did not improve *P. pseudostrobus* performance in our short experiment, since inoculated plants showed negative effects when salts were added. Mycorrhizal inoculum has been shown in other studies to negatively affect growth when phosphorus concentrations are high (Johnson *et al.* 1997, Johnson 1998). In particular, plants with citrate and tartrate showed a decrease in stem diameter while the addition of malate, oxalate and succinate affected plant cover. Mycorrhizae impose an initial cost on young plants, reducing their performance in the short-term during the early stages of colonization as other studies have found, but this initial cost is compensated by strong benefits later on (Pera & Parladé 2005). This ontogenic transition warrants further investigation in our study system with *P. pseudostrobus*.

Addition of organic acids to heavily degraded soils might have a positive direct effect on the plants, as shown by the longer-term responses in our experimental non-inoculated plants. These results are significant since they could be a cheaper and attainable management for restoration purposes. However, the selection of which organic acid to apply is key to having the strongest possible positive effect on plant performance. Therefore, knowledge of the profile of the exudates produced by root systems is desirable. These results could be applicable to restoration efforts in degraded *Pinus* ecosystems.

Acknowledgements

Literature cited

Adeleke R, Nwangburuka C, Oboirien B. 2017. Origins, roles and fate of organic acids in soils: A review. *South African Journal of Botany* **108**: 393-406. DOI: https://doi.org/10.1016/j.sajb.2016.09.002

Ae N, Arihara J, Okada K. 1991. Phosphorus uptake mechanisms of pigeonpea grown in alfisols and vertisols. *In*: Johansen C, Lee KK, Sahrawat KL, eds. *Phosphorus Nutrition of Grain Legumes in the Semi-Arid Tropics*. India: ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), pp. 91-98. ISBN 92-9066-200-X.

Ae N, Arihara J, Okada K, Yoshihara T, Johansen C. 1990. Phosphorus uptake by pigeonpea and its role in cropping systems of the Indian sub-continent. *Science* **248**: 477-480. DOI: https://doi.org/10.1126/science.248.4954.477

Ahonen-Jonnarth U, Van Hees P A W, Lundström U S, Finlay R D. 2000. Organic acids produced by mycorrhizal *Pinus sylvestris* exposed to elevated aluminium and heavy metal concentrations. *New Phytologist* **146**: 557-567. DOI: https://doi.org/10.1046/j.1469-8137.2000.00653.x

Álvarez JF. 2009. *Ecología de micorrizas arbusculares y restauración de ecosistemas*. México: Las Prensas de Ciencias. Universidad Nacional Autónoma de México. ISBN: 978-607-02-3291-6

Baltazar M, Correia S, Guinan K, Sujeeth N, Braganza R, Gonzalves B. 2021. Recent advances in the molecular effects of biostimulants in plants: An overview. *Biomoleculas* **11**: 1096. DOI: https://doi.org/10.3390/biom11081096

Banik S, Dey BK. 1982. Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms. *Plant Soil* **69**: 353-364. DOI: https://doi.org/10.1007/BF02372456

Barroetaveña C, Bassani V, Rajchenberg M. 2012. Mycorrhizal inoculation of *Pinus ponderosa* in Patagonia, Argentina: rootlets colonization, morphotypes descriptions and seedling growth nursery. *Bosque* **33**: 163-169.

Bautista-Hernández S, Raymundo T, Aguirre-Acosta E, Contreras-Pacheco M, Romero-Bautista L, Valenzuela R. 2018. Agaricomycetes gasteroides del bosque mesófilo de montaña de la Huasteca Alta Hidalguense, México. *Acta Botanica Mexicana* **123**: 21-36. DOI: https://doi.org/10.21829/abm123.2018.1266

Bearden BN. 2001. Influence of arbuscular mycorrhizal fungi on soil structure and soil water characteristics of vertisols. *Plant and Soil* **229**: 245-258. DOI: https://doi.org/10.1023/A:1004835328943

Becerril-Navarrete A, Gómez-Romero M, Lindig-Cisneros R, Blanco-García JA, Villegas J, Pineda-García F. 2022. Interacciones biológicas en la restauración: el caso de *Tecoma stans* (Bignoniaceae) y hongos micorrícicos. *Acta Botanica Mexicana* **129**: e1937. DOI: https://doi.org/10.21829/abm129.2022.1937

Bethlenfalvay GJ. 1982. Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: Development of the host plant. *Phytopathology* **72**: 889-893. DOI: https://doi.org/10.1094/Phyto-72-889

Bücking H. 2004. Phosphate absorption and efflux of three ectomycorrhizal fungi as affected by external phosphate, cation and carbohydrate concentrations. *Mycological Research* **108**: 599-609. DOI: https://doi.org/10.1017/S0953756204009992

Cairney, JWG, Chambers SM. 1997. Interactions between *Pisolithus tinctorius* and its hosts: a review of current knowledge. *Mycorrhiza* **7**: 117-131. https://doi.org/10.1007/s005720050172

Chen HC, Zhang SL, Wu KJ, Li R, He XR, He DN, Huang C, Wei H. 2020. The effects of exogenous organic acids on the growth, photosynthesis and cellular ultrastructure of *Salix variegata* Franch under Cd stress. *Ecotoxicology and Environmental Safety* **187**: 109790. DOI: https://doi.org/10.1016/j.ecoenv.2019.109790

Choi DS, Quoreshi AM, Maruyama Y, Jin HO, Koike T. 2005. Effect of ectomycorrhizal infection on growth and photosynthetic characteristics of *Pinus densiflora* seedlings grown under elevated CO2 concentrations. *Photosynthetica* **43**: 223-229 DOI: https://doi.org/10.1007/s11099-005-0037-7

Chuyong GB, Newbery DM, Songwe NC. 2000. Litter nutrients and retranslocation in a central African rain forest dominated by ectomycorrhizal trees. *New Phytologist* **148**: 493-510. DOI: https://doi.org/10.1046/j.1469-8137.2000.00774.x

Eldhuset TD, Swensen B, Wickstrøm T, Wollebæk G. 2007. Organic acids in root exudates from Picea abies seedlings influenced by mycorrhiza and aluminum. *Journal of Plant Nutrition and Soil Science* **170**: 645-648. DOI: https://doi.org/10.1002/jpln.200700005

Ferrera Cerrato R, Alarcón A. 2001. La microbiología del suelo en la agricultura sostenible. *Ciencia Ergo Sum* **8**:175-183.

Frank K, Beegle D, Denning J. 1998. Phosphorous. *In*: Staton MAE, ed. *Recommended Chemical Soil Test Procedures for the North Central Region*. Missouri Agricultural Experiment Station. SB 1001. https://extension.missouri.edu/publications/sb1001#Topics (accessed January 4th 2024)

García-Oliva F. 2005. Algunas bases del enfoque ecosistémico para la restauración. *In*: Sánchez O, Peters E, Márquez-Huitzil R, Vega E, Portales G, Valdés M, Azuara D, eds. *Temas Sobre Restauración Ecológica*. México: Secretaría de Medio Ambiente y Recursos Naturales, Instituto de Ecología, U.S. Fish and Wildlife Service, Unidos para la Conservación A. C. pp 101-111. ISBN: 968-817-724-5

Gómez-Romero M, Lindig-Cisneros R, del-Val E. 2015. Efecto de la sequía en la relación simbiótica entre *Pinus pseudostrobus y Pisolithus tinctorius*. *Botanical Sciences* **93**: 731-740. DOI: https://doi.org/10.17129/botsci.193

Gómez-Romero M, Villegas J, Sáenz-Romero C, Lindig-Cisneros R. 2013. Efecto de la micorrización en el establecimiento de *Pinus pseudostrobus* en cárcavas. *Madera y Bosques* **19**: 51-63. DOI: https://doi.org/10.21829/myb.2013.193327

Graham JH, Eissenstat DM, Drouillard DL. 1991. On the relationship between a plant's mycorrhizal Dependency and rate of vesicular-arbuscular mycorrhizal colonization. *Functional Ecology* **5**: 773-779. DOI: https://doi.org/10.2307/2389540

Grant C, Bittman S, Montreal M, Plenchette C, Morel C. 2005. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian Journal of Plant Science* **85**: 3-14. DOI: https://doi.org/10.4141/P03-182

Guo D, Ali A, Ren C, Du J, Li R, Lahori AH, Xiao R, Zhang Z, Zhang Z. 2019. EDTA and organic acids assisted phytoextraction of Cd and Zn from a smelter contaminated soil by potherb mustard (*Brassica juncea* Coss) and evaluation of its bioindicators. *Ecotoxicology and Environmental Safety* **167**: 396-403. https://doi.org/10.1016/j.ecoenv.2018.10.038

Gyaneshwar P, Kumar GN, Parekh LJ. 1998. Effect of buffering on the phosphate-solubilizing ability of microorganisms. *World Journal of Microbiology and Biotechnology* **14**: 669-673. Doi: https://doi.org/10.1023/A:1008852718733

Hekstra A. 1996. Sustainable nutrient management in agriculture. Amsterdam: ECOSCRIPT-57 Foundation for Ecodevelopment. ISBN: 907-111-172-5

Iyer B, Rajput MS, Rajkumar S. 2017. Effect of succinate on phosphate solubilization in nitrogen fixing bacteria harboring chickpea and their effect on plant growth. *Microbiological Research* **202**: 43-50. DOI: https://doi.org/10.1016/j.micres.2017.05.005

Janos DP. 2007. Plant responsiveness to mycorrhizas differs from dependence upon mycorrhizas. *Mycorrhiza* **17**: 75-91 DOI: https://doi.org/10.1007/s00572-006-0094-1

Johan PD, Ahmed OH, Omar L, Hasbullah NA. 2021. Phosphorus Transformation in Soils Following Co-Application of Charcoal and Wood Ash. *Agronomy* **11**: 2010. DOI: https://doi.org/10.3390/agronomy11102010

Johansson E M, Fransson PM, Finlay RD, van Hees PA. 2008. Quantitative analysis of root and ectomycorrhizal exudates as a response to Pb, Cd and As stress. *Plant and Soil* **313**: 39-54. DOI: https://doi.org/10.1007/s11104-008-9678-1

Johansson EM, Fransson PMA, Finlay RD, Van Hees PAW. 2009. Quantitative analysis of soluble exudates produced by ectomycorrhizal roots as a response to ambient and elevated CO2. Soil Biology and Biochemistry **41**: 1111-1116. DOI: https://doi.org/10.1016/j.soilbio.2009.02.016

Johnson NC. 1998. Responses of *Salsola kali* and *Panicum virgatum* to mycorrhizal fungi\ phosphorus and soil organic matter: implications for reclamation. *Journal of Applied Ecology* **35**: 86-94. DOI: https://doi.org/10.1046/j.1365-2664.1998.00277.x

Johnson NC, Graham J H, Smith FA. 1997. Functioning of mycorrhizal associations along the mutualism-parasitism Continuum. *The New Phytologist* **135**: 575-586. DOI: https://doi.org/10.1046/j.1469-8137.1997.00729.x

Johnson JF, Vance CP, Allan DL. 1996. Phosphorus deficiency in *Lupinus albus*: Altered lateral root development

and enhanced expression of phosphoenolpyruvate carboxylase. *Plant Physiology* **112**: 31-41. DOI: https://doi.org/10.1104/pp.112.1.31

Junk A, Seeling B, Gerke J. 1993. Mobilization of different phosphate fractions in the rhizosphere. *In*: Barrow NJ, ed. *Plant Nutrition from Genetic Engineering to Field Practice*. Dordrecht: Kluwer Academic Publishers, pp. 95-98. ISBN: 978-94-010-4832-3

Li HY, Zhu YG, Marschner P, Smith FA, Smith SE. 2005. Wheat responses to arbuscular mycorrhizal fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply. *Plant Soil* **277**: 221-232. DOI: https://doi.org/10.1007/s11104-005-7082-7

Liang Y, Xiao X, Guo Z, Peng C, Zeng P, Wang X. 2021. Co-application of indole-3-acetic acid/gibberellin and oxalic acid for phytoextraction of cadmium and lead with *Sedum alfredii* Hance from contaminated soil. *Chemosphere* **285**: 131420. DOI: https://doi.org/10.1016/j.chemosphere.2021.131420

Marschner H, Dell B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil* **159**: 89-102. DOI: https://doi.org/10.1007/BF00000098

Meier IC, Avis PG, Phillips RP. 2013. Fungal communities influence root exudation rates in pine seedling. *Fems Microbiology Ecology* **83**: 585-595. DOI: https://doi.org/10.1111/1574-6941.12016

Ohno T, Kubicki JD. 2020. Adsorption of organic acids and phosphate to an iron (oxyhydr)oxide mineral: A combined experimental and density functional theory study. *The Journal of Physical Chemistry A* **124**: 3249-3260. DOI: https://doi.org/10.1021/acs.jpca.9b12044

Peñaloza E, Carvajal N, Corcuera L, Martínez J. 2000. Exudación de citrato y actividad de la enzima fosfoenolpiruvato carboxilasa en raíces de Lupino blanco en respuesta a variaciones en la disponibilidad de fósforo. *Agricultura Técnica* **60**: 89-98. DOI: https://doi.org/10.4067/S0365-28072000000200001

Pera J, Parladé J. 2005. Inoculación controlada con hongos ectomicorrícicos en la producción de planta destinada a repoblaciones forestales: estado actual en España. *Investigación Agraria, Sistemas y Recursos Forestales* **14**: 419-433.

Pérez-Moreno J, Read DJ. 2004. Los hongos ectomicorrízicos, lazos vivientes que conectan y nutren a los árboles en la naturaleza. *Interciencia* **29**: 239-247.

R Core Team. 2020. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, https://www.R-project.org/

Salisbury BF, Ross WC. 1994. *Fisiología vegetal*. DF, México: Editorial Iberoamericana. ISBN: 978-84-282-0212-1

Sakouhi L, Kharbech O, Massoud M, Munemasa S, Murata Y, Chaoui A. 2022. Oxalic acid mitigates cadmium toxicity in *Cicer arietinum* L. germinating seeds by maintaining the cellular redox homeostasis. *Journal of Plant Growth Regulation* **41**: 697-709. DOI: https://doi.org/10.1007/s00344-021-10334-1

Sebastian A, Prasad MNV. 2018. Exogenous citrate and malate alleviate cadmium stress in *Oryza sativa* L.: Probing role of cadmium localization and iron nutrition. *Ecotoxicology and Environmental Safety* **166**: 215-222. https://doi.org/10.1016/j.ecoenv.2018.09.084

Shen H, Yan X, Zhao M, Zheng S, Wang X. 2002. Exudation of organic acids in common bean as related to mobilization of aluminum- and iron-bound phosphates. *Environmental and Experimental Botany* **48**: 1-9. https://doi.org/10.1016/S0098-8472(02)00009-6

Sheng XF, He LY. 2006. Solubilization of potassium-bearing minerals by a wild-type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian Journal of Microbiology* **52**: 66-72. DOI: https://doi.org/10.1139/w05-117

Sheng M, Tang M, Zhang F, Huang Y. 2011. Influence of arbuscular mycorrhiza on organic solutes in maize leaves under salt stress. *Mycorrhiza* **21**: 423-430. DOI: https://doi.org/10.1007/s00572-010-0353-z

Shi S, Condron L, Larsen S, Richardson AE, Jones E, Jiao J, O'Callaghan M, Stewart A. 2011. *In situ* sampling of low molecular weight organic anions from rhizosphere of radiata pine (*Pinus radiata*) grown in a rhizotron system. *Environmental and Experimental Botany* **70**: 131-142. DOI: https://doi.org/10.1016/j.envexpbot.2010.08.010

Smith SE, Read DJ. 2008. Mycorrhizal symbiosis. Cambridge, England: Academic press. ISBN: 9780123705266

Strullu-Derrien C, Selosse M, Kenrick P, Martin F. 2018. The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. *New Phytologist* **220**: 1012-1030. DOI: https://doi.org/10.1111/nph.15076

Tian ZM. 2004. The response of *Lupinus albus* Roots to the signal from phosphorus-deficient substrate. *Russian Journal of Plant Physiology* **51**: 396-401. DOI: https://doi.org/10.1023/B:RUPP.0000028687.22128.60

Tisdall JM. 1994. Possible role of soil microorganisms in aggregation in soils. *Plant and Soil* **159**: 115-121. https://doi.org/10.1007/BF00000100

van der Heijden MGA, Martin FM, Selosse MA, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist* **205**: 1406-1423. https://doi.org/10.1111/nph.13288

Vyas P, Gulati A. 2009. Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent *Pseudomonas*. *BMC Microbiology* **9**: 1-15. DOI: https://doi.org/10.1186/1471-2180-9-174

Wang YN, Wang MK, Zhuang SY, Tu TC, Chiang KY. 2007. Characterization of low-molecular-weight organic acids and organic carbon of Taiwan red cypress, peacock pine, and moso bamboo in a temperate rain forest. *Communications in Soil Science and Plant Analysis* **38**: 77-91. DOI: https://doi.org/10.1080/00103620601093686

Zhang S, Chen H, He D, He X, Yan Y, Wu K, Wei H. 2020. Effects of exogenous organic acids on Cd tolerance mechanism of *Salix variegata* Franch. under Cd stress. *Frontiers in Plant Science* **11**: 594352. DOI: https://doi.org/10.3389/fpls.2020.594352