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Microbial inoculation of *Parkinsonia praecox* (Ruiz & Pav. ex Hook.) Hawkins for ecological restoration

Inoculación microbiana de *Parkinsonia praecox* (Ruiz & Pav. ex Hook.) Hawkins para restauración ecológica

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Abstract. The use of microbial inocula for the restoration of severely degraded arid environments is of great interest. This is because there is a presumption that growth-favoring microorganisms can facilitate the establishment of seedlings by increasing their ability to withstand both the stress of transplantation in the case of nursery seedlings, and the extreme conditions for natural establishment imposed by the scarce, random and variable rainfall. In this work we analyze the "slenderness index"- the height of the plant divided by its diameter at the height cotyledon node- to analyze the effect of treatments with microorganisms in the nursery. This variable is a very feasible criterion for the selection of seedlings for ecological restoration because it is based on information that is easily obtainable in local nurseries. We evaluated this index in nursery seedlings of *Parkinsonia praecox* (Ruiz & Pav. ex Hook.) Hawkins, applying the following treatments: inoculation with the nitrogen-fixing, symbiotic bacterium *Bradyrhizobium japonicum* (TB), inoculation with the arbuscular mycorrhizal fungus *Glomus intraradices* (TM), inoculation with a mixture of *B. japonicum* and *G. intraradices* (TBM), and a control with no inoculation (C). We used top soil of mounds from the Monte ecosystem as a base substrate. It is concluded that the microbial inoculation of native soils of mounds does not provide significant results applicable to the selection of nursery seedlings for ecological restoration in this species. The possible importance of the native soil and the inoculation of the microorganisms in the survival and growth of the species are discussed.

Keywords: Arbuscular mycorhizae; *Bradyrhizobium*; Ecological restoration; Nursery gardens; Top soil.

Resumen. El uso de inóculos microbianos para la restauración de ambientes áridos severamente degradados es de gran interés. Esto se debe a que existe la presunción de que los microorganismos que favorecen el crecimiento pueden facilitar el establecimiento de plántulas al proporcionar la capacidad de soportar tanto el estrés de la plantación, en el caso de las plántulas procedentes de vivero, como las condiciones extremas para el establecimiento natural impuesto por la lluvia escasa, aleatoria y variable. En este trabajo se analizó el índice de esbeltez, formado por el cociente entre la longitud de la planta y su diámetro a la altura del nudo del cotiledón, para evaluar el efecto de tratamientos con microorganismos en el vivero. Esta variable es muy adecuada para la práctica de la restauración ecológica porque proporciona información fácilmente obtenible en viveros locales para la selección de plántulas. Evaluamos este índice en plántulas de vivero de *Parkinsonia praecox* (Ruiz & Pav. ex Hook.) Hawkins, a los que se aplicaron los siguientes tratamientos: inoculación de bacterias simbióticas fijadoras de nitrógeno *Bradyrhizobium japonicum* (TB), inoculación de hongos micorrízicos arbusculares *Glomus intraradices* (TM), inoculación de la mezcla *B. japonicum* con *G. intraradices* (TBM) y control sin inoculación (C). Se utilizó como sustrato base el suelo nativo superficial de montículos del ecosistema del Monte. Concluimos que la inoculación microbiana en suelo nativo superficial en esta especie no proporciona resultados significativos aplicables a la selección de plántulas de vivero para restauración ecológica. Se discute la posible importancia del suelo nativo y la inoculación de los microorganismos en la supervivencia y crecimiento de la especie.

Palabras clave: Micorrizas arbusculares; *Bradyrhizobium*; Restauración ecológica; Suelo superficial; Vivero.

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INTRODUCTION

The importance of the inoculation of microorganisms on seedlings destined to restoration of arid environments has been recognized and evaluated in numerous investigations (Allen et al., 2003; Cáceres & Cuenca, 2006; Fajardo et al., 2011; Hernández-Cuevas et al., 2012). Soils in arid zones frequently contain inocula suitable for mycorrhization and nitrogen fixation (Dias et al., 1995; Herman, 2000). Specifically in arid biotopes like stabilized dunes, the importance of these inocula and symbiosis was tested for Corkidi & Rincón (1997) who reported that 97% of the plant species that inhabit these environments were mycorrhizal. At the same time, in several cases, inoculation with growth-favoring microorganisms promoted greater biomass development in seedlings destined to restore degraded arid environments with stressful conditions (Solís-Domínguez et al., 2011; Ortiz et al., 2015).

When choosing seedlings for ecological restoration or quality diagnoses of stocks of seedlings in local nursery plants, practical variables with good correlation with the biomass of the seedlings are required. These variables should not have high economic costs or require the destruction of seedlings, to the extent possible. Although height can be one of these variables because it is usually relatively strongly correlated with the amount of organic matter, if the plant grows under stress or non-optimal conditions, the stems can be very thin. To circumvent this issue, the use of the ratio of the height to the stem diameter of the plant, called the "slenderness index" (Orozco Gutiérrez et al., 2010) has been recommended. Intermediate slenderness index values indicate that the plant is neither "stunted" nor "withered" (very low and very high slenderness indexes, respectively). Specifically in restoration it has been found that intermediate slenderness index values produce better plant growth in the field (Villar, 2003).

Inoculation is one of the main and most promising options for achieving optimal growth indexes in nursery seedlings. One of the options to obtain the microorganisms used is the commercial market. Recently Báez-Pérez et al. (2015) obtained positive results with the application of commercial inocula in the establishment of seedlings in severely degraded soils. Another option has been the use of native microorganisms, though slow-growing species showed higher relative growth rates than fast-growing species in tropical, seasonally dry forests species (Huante et al., 2012).

Although microorganisms can play a fundamental role in the growth of several species (Barreto et al., 2013), it should be considered that dryland species early in succession likely depend little on these microorganisms. This is because they are able to compensate the drought, extremely low resource availability and low proportion of microbian inocula with other physiological capacities (Abella, 2010; Villagra et al., 2011). The adaptations to drought reported in arid zones are very varied. They include, for example, stomatal closure to avoid water loss, reduction of leaf area, high foliar reflectance due to high density of trichomes (Larcher, 2000; Valladares et al., 2004), reduction of incident radiation by foliar movements (Ehleringer & Cooper, 1992) and by photoprotection mechanisms (Tezara et al., 2010), maximization of water absorption by increasing the surface area of root uptake (Jackson et al., 2000), development of deep root systems (Valladares et al., 2004), optimization of carbon gain during unfavorable periods from photosynthetic stems (Nilsen, 1995; Ávila et al., 2014a), succulence, high efficiency of water use, and osmotic adjustments (Ávila et al., 2014b). *Parkinsonia praecox* is an example of a species that combines numerous ecophysiological adaptations that favor productivity in arid environments. These include have stems capable of photosynthesizing, and slenderness, of fundamental importance in this species. At the same time, this species is mentioned as a colonizer of the most degraded arid biotopes. In these cases, near 100% seedling survival was reported in field experiences of rehabilitation without inoculation with microorganisms (Martinez Carretero, 1986; Pérez et al., 2010; Zúñiga & Pérez, 2014).

In this framework, the purpose of this work was to evaluate the effect of the use of microbial inocula on the slenderness index of *P. praecox* seedlings meant for use in ecological restoration. If this inoculation were effective, the benefit should be manifested in optimal growth, and the seedlings should have intermediate slenderness indexes, while the non- inoculated plants should have more extreme (low or high) slenderness values.

MATERIALS AND METHODS

Species description. *Parkinsonia praecox* (Ruiz & Pav. ex Hook.) Hawkins, also named as *Cercidium praecox* ssp. *glaucum* (Cav.) Burkart & Carter, is a shrub species belonging to the family Fabaceae, subfamily Caesalpinoidea, tribe Caesalpinieae; popularly known as Chañar brea (Burkart & Carter, 1976; Martínez Carretero, 1986; Romão & Mansano, 2018). It has been mentioned that this primary colonizing species is capable of growing in degraded environments, where it usually forms nearly pure stands (Martínez Carretero, 1986). *Parkinsonia praecox* is characteristic of the Monte phytogeographical province. It grows between 650-2,200 m a.s.l., on stony ground of dry or sandy rivers, sometimes associated with *Larrea divaricata* Cav. and *L. cuneifolia* Cav. (Ulibarri, 1997; Ulibarri, 2008).

Collection of seeds and substrate. The seeds were collected in Aguada Pichana (38° 32' 95.35" S, 68° 99' 69.15" W), Añelo, Neuquén, Argentina. Aguada Pichana is located in the Monte phytogeographical region, in its austral region or Monte Austral Neuquino (Busso & Bonvissuto, 2009). It has an arid climate with precipatation that averages 180 mm annually, and droughts from 6 to 9 months (Labraga & Villalba, 2009). The mature fruits of *P. praecox* were collected in January 2011 from 62 individuals and were stored in the Germplasm Bank of the Arid at -4 °C (Rodríguez Araujo et al., 2015). The seeds were cleaned (Fig. 1a), and subjected to a pre-germination treatment of acid scarification (Paredes et al., 2018). The base substrate for the experimentation was obtained from sites located in the same place as seed collection. The collection yielded a composite sample of 100 extractions of 500 cm^3, not exceeding the upper 5 cm of the soil within an area of 1ha.

Substrate analysis. A random sample of the obtained soil was analyzed for texture (Bouyoucos), pH (1: 2.5), electrical conductivity (saturation paste extract), sodicity (flame photometry), organic matter content (Walkey Black), phosphorus (Olsen), nitrogen (Kjeldahl) and interchangeable potassium (extraction with ammonium acetate pH 7).

Seeding. One seed was planted per pot (125 cm^3) at a depth of 1.5 times its size in length. The average seed size was 5.06 ± 0.36 mm wide, 7.48 ± 0.51 mm long, 2.28 ± 0.24 mm thick, and 0.060 ± 0.0036 g weight. The substrate was moistened slowly to prevent the seed from sinking or being exposed.

Treatments. Using the native top soil of mounds as substrate, a control treatment without inoculation (C) and three where inoculation was applied: inoculation with the bacterium *Bradyrhizobium japonicum*, trademark Rizobacter® (TB), inoculation with the fungus *Glomus intraradices*, trademark Glomusphit® (TM) and inoculation with a mixture of bacteria and fungi, *Bradyrhizobium japonicum* and *Glomus intraradices* (TBM) (Fig. 1b). The native soil was sandy in texture, slightly alkaline (pH=7.79), slightly saline (3.4 dS/m), and non-sodium (7.41%), with moderate potassium (277 ppm), low percentage of organic matter (0.35%), 4.2 ppm of phosphorus and 0.0175% of total nitrogen.

Inoculation. The inoculum was applied when seeds were sown (Fig. 1c). TB was inoculated with 1 mL of inoculant (1×10^{10} bacteria/mL), TM was inoculated with 1 mL of inoculant (100 spores/mL), and TBM was inoculated with 1 mL of each of the two inoculants. The control (C) was not inoculated.

Fig. 1. Stages of inoculation of *Parkinsonia praecox* in nursery: (a) separation and cleaning of seeds of ripe fruits; (b) conditioning of microbial inocula in the laboratory; (c) inoculation of fungi and bacteria in the substrate at the time of seeding; (d) seedling development in the nursery garden.

Fig. 1. Etapas de la inoculación de *Parkinsonia praecox* en vivero: (a) separación y limpieza de semillas de frutos maduros; (b) acondicionamiento de inóculos microbianos en el laboratorio; (c) inoculación de hongos y bacterias en el sustrato en el momento de la siembra; (d) desarrollo de plántulas en vivero.

Experimental conditions. The average temperature in the nursery garden was 16.68 °C. The photoperiod during the emergence period was 10.5 hours light and 13.5 hours darkness. Irrigation was provided twice a day for 5 minutes to maintain field capacity.

Data analysis. The response variable was the ratio of plant height to the diameter of the stem at the height of the cotyledon node at 108 days after the emergence of cotyledons (Fig. 1d). The number of specimens (n) in each treatment group was between 16 and 26, depending on survival of the plants in each group. Differences among treatments were evaluated using a one-way analysis of variance (ANOVA) and Tukey test with a level of significance of $P<0.05$. The statistical analysis was performed using STATISTICA 7.1 (StatSoft, 1984-2006).

RESULTS

No nitrogen fixation nodules were observed in the roots of *P. praecox* in any of the treatments. No significant differences were found in the slenderness index among the applied inoculation treatments (Fig. 2).

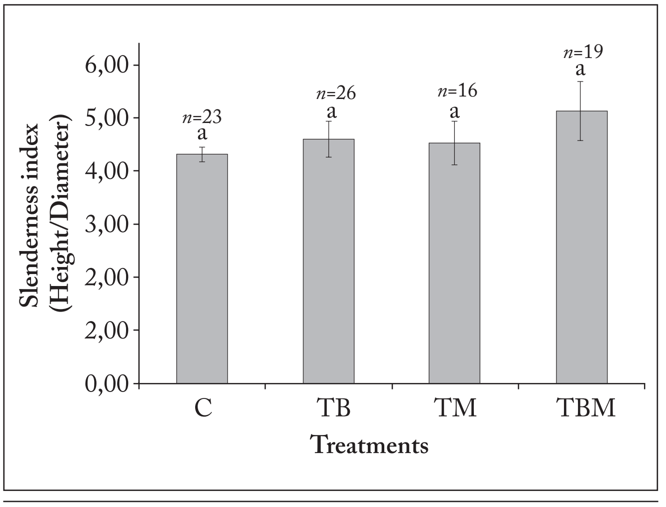

Fig. 2. Slenderness index measured at 108 days in non-inoculated control plants (C), plants inoculated with the bacterium *B. japonicum* bacteria (TB), with the mycorrhizal fungus *G. intraradice* (TM) and bacterial and fungal inocula combined (TBM). The bars represent the mean of each treatment, and the error bars indicate the standard deviation. Values that share a letter are not significantly different (P<0.05), by one-way ANOVA.

Fig. 2. Índice de esbeltez medido a los 108 días de plantas de control no inoculadas (C), plantas inoculadas con bacterias de *B. japonicum* (TB), con hongos micorrízicos *G. intraradices* (TM) y combinación de inóculo bacteriano y fúngico (TBM). Las barras representan la media de cada tratamiento, y las barras de error indican la desviación estándar. Los valores que tienen una letra común no son significativamente diferentes (P<0,05), por ANOVA de una vía.

DISCUSSION

When planting *P. praecox* for restoration purposes in severely degraded environments of the Monte with nursery specimens, different heights and seedling diameters have been shown to have differences in survival (Zúñiga & Pérez, 2014). The specimens considered "small" (8.83 ± 2.67 cm in height and 3.39 ± 0.60 mm in diameter) performed more poorly, both with and without hydrogel, than the "large" specimens (16.73 cm ± 4.32 heights and 4.20 mm ± 0.73 in diameter) (Pérez et al., 2010). These results show that the slenderness attribute correlates with the success of restoration plantings in this species. In this study we found that inoculation with microorganisms does not modify this useful attribute for the selection of nursery seedlings.

Unlike other Caesalpinioideae colonized by *Bradyrizhobium* (Andrews & Andrews, 2017), the presence of nodules in the roots of *P. praecox* was not detected. This is consistent with studies that indicate that numerous drought-tolerant fabaceae or colonizing species are not able to nodulate or do not respond to inoculation with changes in growth-related variables (Sprent, 1987; Huante, 2012). Also, Allen & Allen (1961) pointed out the low nodulation capacity of the subfamily Caesalpinioideae in comparison with the subfamilies Mimosoideae and Papilionoideae.

An important aspect to consider in the use of substrates for restoration is the possible effect of the transfer of both microorganisms and exotic seeds or seedlings that can interfere with the recovery process of degraded sites. The use of native top soil has been strongly advised in the restoration literature (Clewell & Aronson, 2013). In our study we found that the native soil may be suitable for the growth of seedlings for restoration and that the addition of allocthonous microorganisms does not produce detectable benefits measurable through the slenderness variable in the aereal parts of the plants.

The present work is the first contribution to the relationship of microorganisms with the growth of *P. praecox* in the nursery, so the existence of specificity of associations between microorganisms and the roots of this species and possible effects on biomass and roots should be further explored. It is also necessary to confirm mycorrhizal colonization with tissue analysis and to evaluate differences between degraded soils and mound soils, and the possible competitive effect between native and non-native microorganisms in order to determine if there are particular concentrations of inocula that could provide measurable effects in the aerial part of this species.

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