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TRABAJO CIENTÍFICO

Germination of three Fabaceae species of interest for ecological restoration in the Southern Monte, Patagonia, Argentina

Germinación de tres especies de Fabaceae de interés para la restauración ecológica en el Monte Austral, Patagonia, Argentina.

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RESUMEN

Grandes áreas del Monte Austral han sido severamente degradadas por el pastoreo excesivo y actividades de extracción de petróleo. La rehabilitación y la restauración a través de la plantación de especies nativas se consideran alternativas adecuadas para revertir este problema. En particular se recomiendan plantaciones de especies de Fabaceae para reiniciar la sucesión ecológica porque pueden mejorar las propiedades físicas y químicas de los suelos y por su capacidad para fijar nitrógeno. Además, esta familia incluye numerosas especies que proporcionan forraje, madera, ornamentación y materiales con potencial uso industrial, y pueden incluirse en aquellos proyectos de restauración que integren objetivos sociales y ecológicos. Para explorar la aptitud de estas especies en la rehabilitación y restauración de áreas degradadas, inicialmente es necesario evaluar qué tratamientos pregerminativos son apropiados para una germinación rápida y uniforme de las semillas en vivero. En este contexto, el objetivo de nuestro estudio fue determinar el efecto de los tratamientos de escarificación química (durante 5 y 45 minutos) y los tratamientos húmedos y fríos (durante 7 y 30 días) sobre la germinación de las siguientes especies: *Parkinsonia praecox (Chañar brea)*, *Prosopidastrum striatum (Manca caballos)* y *Senna aphylla (Pichanilla)*. Las tres especies estudiadas tuvieron mayores porcentajes de germinación con escarificación química durante 45 minutos, mientras que los tratamientos húmedo-frío no difirieron del control. Concluimos que la escarificación ácida es adecuada para la propagación a gran escala de estas especies, ya que permite tratar grandes cantidades de semillas en poco tiempo y con resultados homogéneos.

Palabras clave: *Parkinsonia praecox, Prosopidastrum striatum,* semillas, *Senna aphylla,* tratamientos pregerminativos.

ABSTRACT

Large areas of the Southern Monte in the Patagonia have been severely degraded by overgrazing and oil extraction activities. Rehabilitation and restoration by planting native species are suitable alternatives to reverse this problem. Planting Fabaceae species is particularly recommended to reinitiate the ecological succession because of their ability to improve the soil physical and chemical properties as well as their nitrogen-fixing capability. In addition, this family includes several species that are used as fodder, timber, and for decorative and industrial uses. Thus, these species can be included in restoration projects that integrate social and ecological goals. To explore the suitability of these species for the restoration and rehabilitation of degraded areas, it is necessary to assess what pre-germination treatments are appropriate to obtain a fast and uniform germination in nursery gardens. The goal of this study was to determine the effect of chemical scarification treatments (5 or 45 minutes) and wet-cold treatments (7 or 30 days) on the germination of the following species: *Parkinsonia praecox*, *Prosopidastrum striatum*, and *Senna aphylla*. The three studied species showed the highest germination percentages with chemical scarification during 45 minutes, while the wet-cold treatments did not differ from the control. In conclusion, the acid scarification treatment is suitable for the large-scale propagation of these species because a substantial quantity of seeds can be treated in a short time and with homogeneous results.

Key words: *Parkinsonia praecox, Prosopidastrum striatum,* seeds, *Senna aphylla,* pre-germination treatments.

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1. INTRODUCTION

The Monte comprises 50 million hectares and is considered the most arid region of Argentina (Abraham *et al*., 2009). Due to ecological differences throughout its great extension, it is divided into three districts: Northern, Eremean, and Southern (Roig, 2009). The Southern District is located south of the Colorado River and is characterized by a landscape of Patagonian plateaus and scarcity of trees (Movia *et al*., 1982). Unfortunately, the Southern Monte has been degraded by overgrazing and oil extractive activities (Guevara *et al*., 1997; Busso and Fernández, 2017). As a result, areas with bare soil, landscape fragmentation, and desertification have increased (Fiori and Zalba, 2003; Mazzonia and Vazquez, 2009).

Active restoration works by planting native species are needed to reverse the degradation of the Monte (Bertiller and Bisigato, 1998; Pérez *et al*., 2011). One of the plant families recommended to restore degraded areas in arid zones is the Fabaceae (Aronson *et al*., 1993; Bainbridge, 2007; Padilla *et al*., 2009) because many species of this family can improve the physical and chemical properties of soils and can fix nitrogen from the atmosphere (Chinea *et al*., 2004, Córdova-Sánchez *et al*., 2011, Castellano-Hinojosa *et al*., 2016). Different authors highlighted the importance of this plant family to facilitate ecological succession (Van Andel *et al*., 1993) and to achieve a self-sustaining recovery of arid environments (Marrs *et al*., 1981). In addition to its ecological contribution, this family includes numerous species that provide fodder, timber, and materials for decorative and industrial purposes (Burkart, 1952; Lewis *et al*., 2005; Barboza *et al*., 2009). Thus, they can be used in productive restoration projects that pursue both ecological and social goals (Ceccon, 2013). However, restoration projects in large desertified areas of the Monte in the Patagonia require many seedlings, for which different propagation techniques can be used, such as sexual reproduction from seeds, division of vegetative parts, and seedling rescue. The importance of using seeds lays in their genetic variability, which confers a higher likelihood of survival through the process of natural selection (Gold *et al*., 2004; Fenner and Thompson, 2005). Germination treatments for certain Fabaceae from arid zones of Argentina showed dissimilar results according to different authors and the origin of the seeds (Funes and Venier, 2006; Masini *et al*., 2012; Galindez *et al*., 2016; Rodriguez Araujo *et al*., 2017; Zapata *et al*., 2017). In particular, in the Southern Monte, the results of pre-germination treatments showed significant variations in the germination percentage of *Ephedra ochereata* Miers even throughout distances of approximate 500 km (Rodríguez Araujo *et al*., in review). For other Fabaceae species from Southern Monte and Patagonia, the most effective duration of chemical scarification treatments differed according to the biogeographical origin of the species (Rodríguez Araujo *et al*., 2017).

In this paper, we assessed the effectiveness of different germination treatments for three species of Fabaceae native to the Montee used as livestock fodder and have potential value for ecological restoration (Von Müller *et al*., 2007; Pérez *et al*., 2010; Muiño, 2010): *Parkinsonia praecox* (Ruiz & Pav. ex Hook.) Hawkins; *Senna aphylla* (Cav.) H.S. Irwin & Barneby, and *Prosopidastrum striatum* (Benth.) R.A. Palacios & Hoc. Based on the hypothesis that the effectiveness of pre-germination treatments differs according to the species, the objectives of this study were the following:

- To evaluate the effect of different treatments on the germination of *Parkinsonia praecox*, *Prosopidastrum striatum*, and *Senna aphylla*.
- To assess if prolonged periods under humid conditions allow the germination of the three species; and
- To determine which treatment achieves the highest germination in a fast and uniform way for its application in restoration projects.

2. MATERIALS AND METHODS

Study area

Seed collection was carried out in the Southern Monte, to the east of the Neuquén Province, below 400 m.a.s.l. The average annual rainfall is less than 200 mm, with a maximum rainfall in spring and winter and a minimum in summer (Morello, 1958). The average temperature is 13-14 °C, while the monthly averages of day and night temperatures vary between 28 °C and 15 °C in January and 7 °C and 2 °C in July, respectively. Vegetation occupies 45% of the area and is distributed in oval patches formed by shrubs and grasses (Busso and Bonvissuto, 2009). The dominant shrubs are *Larrea divaricata* Cav., *L. cuneifolia* Cav., *L. nitida* Cav., *Monttea aphylla* (Miers.) Benth. and Hook. var. *aphylla*, *Boungainvillea spinosa* (Cav.) Heimerl, *Atriplex lampa* (Moq.) D. Dietr., *Schinus johnstonii* F. A. Barkley, and *Lycium chilense* Miers ex Bertero. Different species of grasses are commonly associated with these shrubs, such as *Pappostipa speciosa* (Trin. and Rupr.) Romasch, *Eremium erianthum* (Phil.) Seberg & Linde-Laursen, and *Poa ligularis* Nees ex Steud. (Movia *et al*., 1982).

Distribution, main characteristics of the species, and known uses

- *Parkinsonia praecox* (previously called *Cercidium praecox (*Ruiz & Pav. ex Hook.) Harms *ssp. glaucum* (Cav.) Burkart & Carter).

  Distributed in Argentina coincidently with the phytogeographic province of the Monte (Martínez Carretero, 1986), it is a shrub up to 2.5 m high, short trunk, green bark. The flowers are 10-14 mm, yellow, with a small number of flowers in each cluster. The legume measures 3-8 x 0.5-1.5 cm, is ellipsoid to oblong and compressed. The seeds measure 3-4 mm and are ovoid, greenish with brownish streaks (Correa, 1984; Martínez Carretero, 1986). This species lives in extremely arid sites and grows well in rocky cracks and on stony hillsides with up to 80-90% slope (Correa, 1984). It blooms in spring and summer and bears fruits in late summer and early fall. The gum that is exuded from its bark is used for candy and as glue. Due to the rapid decomposition of its wood, it has little application as fuel (Alesso *et al*., 2003).

- *Prosopidastrum striatum*

  It is distributed from the south of Mendoza and the north of Neuquén provinces, in an easterly direction, towards the south of the Buenos Aires province, and along the Patagonian coast up to Santa Cruz province (Palacios and Hoc, 2005). It is a shrub of 1-1.5 m high, with stiff, sharp branches, 2-5 mm in diameter at the base and arranged at 35-90° with respect to their carrier axis. It has capituliform inflorescences, 7-13 mm in diameter, with 15-25 flowers. The dry fruit has a linear to elliptical or oblong outline, dehiscent by the ventral suture, or indehiscent (lomento) that is disarticulated in 3-19 monospermic pods. The seeds have an oval outline and a fissured line interrupted near the micropyle (Palacios and Hoc, 2005). It is used as fodder, especially for horses that prefer hard pastures (Muiño, 2010).

- *Senna aphylla*

  Widely distributed, it is one of the characteristic members of the phytogeographic province of the Monte between 50 and 1,800 m.a.s.l. (Correa, 1984). It is a shrub or sub-shrub of 0.40-1.50 m in height. Branches measure 0.75-4 mm in diameter and are flexible, glabrous, with an untidy appearance, and slightly pendulous ends. The flowers are located in simple clusters arranged along the top of the branches, with yellow-orange petals. The fruit is a straight or slightly curved legume with seeds in oblique longitudinal position (Correa, 1984; Gandullo *et al*., 2004). It is used as natural fodder in arid zones, firewood, and for manufacturing brooms, fences, and rural roofs; it is also used as a medicinal and ornamental plant (Gruneisen, 1996; Palacio, 2010).

Collection and processing

The site and date of collection was different for each species since the legumes were obtained directly from the plants when they reached maturity (Table 1). Collecting protocols were followed to ensure a representative sample of the genetic variability, which implied collecting at least 30 healthy plants without exceeding 20% of mature fruits per plant at the time of collection (Gold *e al*., 2004; Ulian *et al*., 2008). The seeds collected from each species were gathered in a set (seed pool) for the experiments.

Then, the seeds were stored in the Germplasm Bank of the “Árido” (Rodríguez Araujo *et al*., 2015) in a controlled freezer at -8 °C until the time of the experiment. This temperature is within the range recommended by Johnson (1983) to maintain seed viability and prevent insect damage in other Fabaceae.

Table 1. Collecting sites and dates

Species	Origin	Coordinates	Collecting dates	Germination testing dates
Parkinsonia praecox	Aguada San Roque	38°00´00.60" South 68°55´22.03" West	December 2010	June 2011
Prosopidastrum striatum	Dique Planicie Banderita	38°36´30.78" South 68°24´43.20" West	January 2013	May 2013
Senna aphylla	Aguada San Roque	38°00´00.60" South 68°55´22.03" West	December 2010	June 2011

Germination treatments

Two chemical scarification times were applied using sulfuric acid: 5 minutes (Ch5) and 45 minutes (Ch45). Likewise, two wet-cold treatments were tested: 7 days (Wc7) and 30 days (Wc30), together with a control group without any treatment (C). For the chemical scarification treatment (Ch5 and Ch45), seeds of each species were placed in beakers, and 98 % sulfuric acid was added until they were completely covered. The mixture was periodically stirred with a glass rod for the time corresponding to each treatment. Then, the mixture was drained in a plastic strainer placed on a funnel to collect the acid waste in a suitable container for storage until its final disposal. The seeds were rinsed with running water and placed on blotting paper.

For the wet-cold treatments (Wc7 and Wc30), plastic trays covered with a cotton layer moistened with water were used. A layer of paper napkins was placed on top of the cotton to hold the seeds. A new layer of napkins was placed on top of the seeds, followed by another layer of cotton. Subsequently, the cotton was sprayed with a fungicide to prevent fungal growth (Willan, 2000). Finally, the layers were covered with another plastic tray, and the whole sets were placed in a refrigerator at 4 °C and in the dark for the duration of each treatment.

Germination assays

For the germination tests, the seeds were placed in Petri dishes of 9 cm in diameter with filter paper. Subsequently, they were placed in a germination chamber at a minimum temperature of 10 °C ± 1 °C during 12 hours of dark and at a maximum temperature of 20 °C ± 1 °C during 12 hours of light, which represents the conditions in which the seeds would be during autumn germination (Paez *et al.*, 2005). Germination (i.e., the emergence of the radicle) was recorded every other day for a period of 42 days, and the germinated seeds were removed from the box.

To assess the effect of the treatments, the germination percentage at the end of the experiment and the average germination times (AGT) were calculated. The AGT is the average number of days a single seed takes to germinate, calculated by the following formula (Ranal and Santana, 2006):

$$\text{AGT} = \frac{\sum_{i=1}^{n} fi \,.\, xi}{\sum_{i=1}^{n} xi}$$

Where: *fi* is the number of days since the beginning of the germination experiment, and xi is the number of seeds that germinated within consecutive time intervals.

Statistical analysis

A completely randomized design with one factor and five levels (treatments) was used. There were three replicates of 30 seeds each. The data was analyzed with an ANOVA using the InfoStat software. The assumptions of normality were evaluated using the modified Shapiro-Wilk test, and the homogeneity of variance was assessed using the Levene test. The analyses of the germination percentage of *P. striatum* and the AGT of *S. aphylla* were performed using the Kruskal Wallis nonparametric test because the assumption of homogeneity of variance was not met. In all cases, the significance level was 0.05.

3. RESULTS

Germination assays

The three studied species presented the highest germination percentages with the Ch45 treatment: 82.2 % for *P. praecox*, 75.5 % for *P. striatum*, and 100 % for *S. aphylla* (Figure 1). The Ch5 treatment produced intermediate germination values (higher than the control group but lower than those with Ch45) for the species *S. aphylla*. In the case of *P. praecox* and *P. striatum*, Ch5 did not produce significant differences in germination compared to the control. Likewise, the wet-cold treatments (Wc7 and Wc30) did not show significant differences in germination compared to the control for the three studied species.

When analyzing the germination results as a function of time (Figure 2), we found that *P. praecox* rapidly increased the germination percentage with Ch45 after 4 days since the beginning of the experiment (60 %) and peaked (82.2 %) on day 36. In this species, the treatments Ch5, Wc7, Wc30 and the control showed gradual germination throughout the experiment with maximum values of 34.4, 17.8, 17.8 and 20 %, respectively. The species *P. striatum* also responded well to the Ch45 treatment since it reached 50 % germination on day 11 and a maximum of 75 % on day 32. In the Ch5 and control treatments, the seeds germinated on day 14 and reached a value of 18.8 % on day 42 and 4.4 % on day 39, respectively. In the Wc7 and Wc30 treatments, the germination was slower and reached only 5.6 % and 6.7 % on days 30 and 28, respectively. *S. aphylla* germinates in a fast and homogeneous manner with the Ch45 treatment since it exceeded 90% germination 4 days after the beginning of the experiment and reached 100 % on day 8. The response to the Ch5 treatment was similar but with lower germination percentages (83.3 %). Unlike the experimental treatments, the germination of the control was slower and reached only 20 %. The Wc7 and Wc30 treatments were also slower and had germination percentages lower than the control (14.4 %).

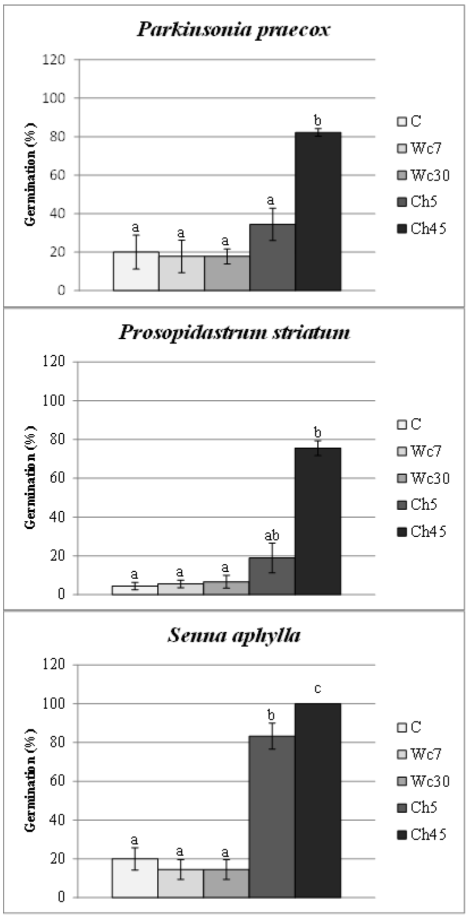

Figure 1. Germination percentage for each species at the end of the experiment (mean and standard deviation) according to each treatment (C: control; Wc7: Wet-cold 7 days; Wc30: Wet-cold 30 days; Ch5: Chemical scarification 5 minutes; Ch45: Chemical scarification 45 minutes). Different letters indicate significant differences at $p<0.05$.

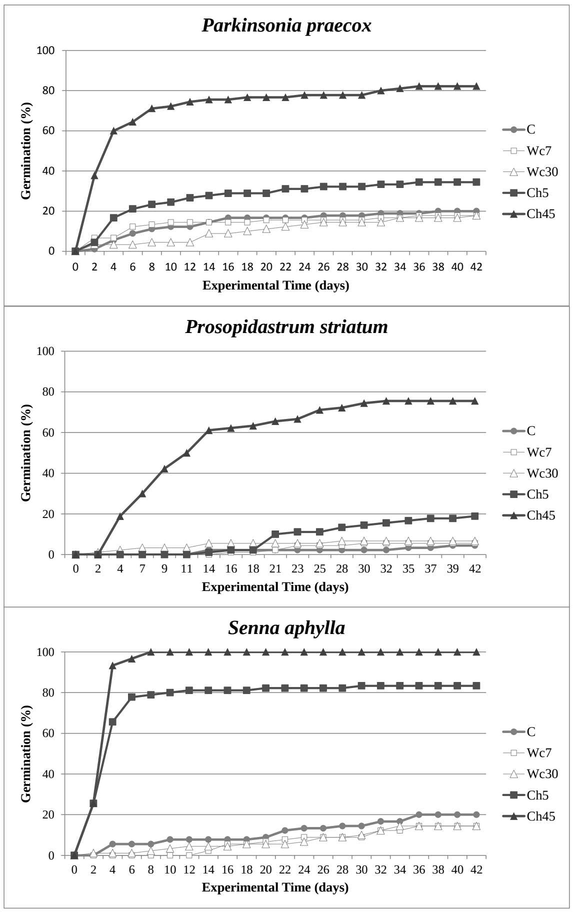

Figure 2. Accumulated germination percentage over time for each species according to each treatment (C: control; Wc7: Wet-cold 7 days; Wc30: Wet-cold 30 days; Ch5: Chemical scarification 5 minutes; Ch45: Chemical scarification 45 minutes).

For *P. praecox* non-significant differences were found in the AGT of the different treatments and the control group. However, the AGT of the Ch45 treatment was significantly lower than that of the Wc30. The AGT of *P. striatum* did not show differences among treatments and control probably due to the high variability of the results. In the case of *S. aphylla*, the Ch45 chemical scarification treatment showed a lower AGT (4.2 days) than Ch5, Wc7, Wc30 and the control (Figure 3).

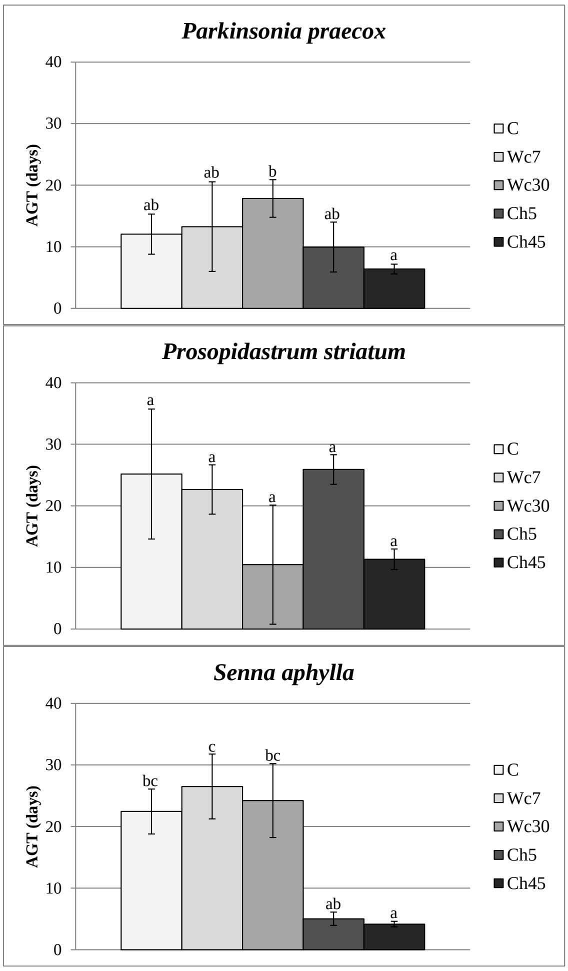

Figure 3. Average Germination Time (AGT) for each species according to each treatment (C: control; Wc7: Wet-cold 7 days; Wc30: Wet-cold 30 days; Ch5: Chemical scarification 5 minutes; Ch45: Chemical scarification 45 minutes).

4. DISCUSSION

The high germination percentages achieved using acid scarification showed the need for carrying out pre-germination treatments to obtain a fast and homogeneous germination. This result is consistent with the need of pre-germination treatments for other species of Fabaceae (Sanabria *et al*., 2001; Pece *et al*., 2013; Campos and Velez, 2015; Galíndez *et al*., 2016). Species, subspecies, varieties, and seed sources constitute critical factors because they can cause significant differences in the outcomes of germination experiments (Cervantes *et al*., 2014; Rodríguez Araujo *et al*., 2017). For instance, research performed in the "Chaco Seco" (northern Argentina) with *Cercidium praecox* subsp. *praecox* (currently named *Parkinsonia praecox*) demonstrated the presence of physical dormancy that, after mechanical scarification (Funes *et al*., 2009) and chemical scarification with sulfuric acid for 3 minutes (Pece *et al*., 2013) resulted in germination percentages over 90 %. These results differ from those obtained in the present study for *Parkinsonia praecox* (previously called *C. praecox* subsp. *glaucum)* in the Southern Monte, where the germination percentage did not exceed 40 % when the acid was left for only 5 minutes. Instead, a high germination percentage was obtained using sulfuric acid for 45 minutes.

Within the genus *Prosopis* (Fabaceae), mixed results have been obtained according to the different origins of the Patagonian species (Vilela and Ravetta, 2001; Pentreath *et al*., 2005; Zeberio and Calabrese, 2013). In the case of *Prosopis alpataco* Phil., the disparity among the reported results with seeds from the Neuquén ranged from the absence of physical dormancy (Galíndez *et al*., 2016) to the lack of germination without scarification (Rodríguez Araujo *et al*., 2017). In the case of *Senna aphylla*, physical dormancy and high germination percentages with mechanical and chemical scarification were also found (Funes, *et al*., 2009; Galíndez *et al*., 2016). In the latter study, using seeds from the Neuquén province without specifying their locality, germination percentages higher than 90% and low AGT were obtained with ten minutes of acid exposure. In this study, similar germination percentages were obtained with 5 and 45 minutes of acid scarification (83.3 % and 100 %, respectively); however, the AGT were higher (5 ± 1.1 days and 4.2 ± 0.4 days, respectively). In other ecosystems, differences in germination have also been found depending on the distance among collection sites (Pérez-Domínguez *et al*., 2013).

In addition, no other study has evaluated the suitability of germination treatments to break the dormancy of *Prosopidastrum striatum*. Thus, the present work constitutes the first assessment of adequate treatments to enable the germination of this species.

The high germination percentages achieved for the three studied species through acid scarification broaden the diversity of species available for restoration projects in the Southern Monte. The reintroduction of seedlings of these Fabaceae species in severely degraded sites can kick-start plant succession, facilitating the establishment and growth of other plant species and contributing to increased soil fertility (Marrs *et al.*, 1981; Gilbert and Anderson, 2003).

5. CONCLUSIONS

Based on these results, it is concluded that the chemical scarification treatment with sulfuric acid during 45 minutes is the best treatment for the three studied species. Acid scarification is applicable for the propagation of species on a large scale because it allows treating large quantities of seeds in a short period of time and with consistent results. Thus, this technique is suitable for ecological restoration projects using both seedlings produced in nursery gardens and in direct seeding assays in the field. Furthermore, the mere presence of moisture for long periods of time does not produce high germination percentages for *P. praecox*, *S. aphylla*, and *P. striatum* from the studied origins.

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