

Germination temperatures and seed dormancy of two *Larrea* species (Zygophyllaceae) from the Monte Desert, Argentina

Temperaturas de germinación y dormición de semillas de dos especies de *Larrea* (Zygophyllaceae) del desierto del Monte, Argentina

María Emilia Fernández ¹, Mariano Anibal Cony ¹, Carlos Bernardo Passera ²

Originales: *Recepción*: 28/03/2019 - *Aceptación*: 30/07/2019

ABSTRACT

The genus *Larrea* includes five species of desert shrubs distributed along the American Continent. These species produce dormant mature seeds, but the type of dormancy and the factors that produce it have been poorly assessed. The objective of this work was to determine the optimum germination temperatures of *L. cuneifolia* and *L. nitida*, to analyze the response to pre-germination treatments, and to evaluate the type of seed dormancy these species have. Seeds were incubated at five constant temperatures and were subjected to mechanical scarification and rinsed with running water to break dormancy. Seed coat permeability and the presence of water-soluble germination inhibitors were also assessed. The optimum germination temperature range was between 15-40°C for both species. A positive response to all pre-germination treatments was observed in *L. cuneifolia* (37-47%), while *L. nitida* showed higher germination percentage only with mechanical scarification (73%). Both species presented water-permeable seed coats, ruling out the occurrence of a physical dormancy. The inhibitory test of seed-coat extracts was positive for *L. cuneifolia*, suggesting the possible presence of a chemical dormancy. These results are valuable for conservation purposes and directly contribute to improving production of seedlings required for restoration projects.

Keywords

germination inhibitors • *Larrea cuneifolia* • *Larrea nitida* • optimum temperature • physical dormancy

1 CCT Mendoza-CONICET. Instituto Argentino de Investigaciones de las Zonas Áridas (IADIZA). Ruiz Leal s/n. Parque Gral. San Martín. CC 507. Mendoza 5500. Argentina. * mefernandez@mendoza-conicet.gob.ar

2 Universidad Nacional de Cuyo. Facultad de Ciencias Agrarias. Cátedra de Fisiología Vegetal. Almirante Brown 500. Chacras de Coria - Luján de Cuyo. CPA M5528AHB. Mendoza. Argentina.

RESUMEN

El género *Larrea* incluye cinco especies de arbustos desérticos distribuidos a lo largo del Continente Americano. Estas especies producen semillas en estado de dormición, pero el tipo de latencia y los factores que la producen han sido poco estudiados. El objetivo de este trabajo fue determinar las temperaturas óptimas de germinación de dos especies de *Larrea*, analizar la respuesta a los tratamientos pre-germinativos y evaluar el tipo de dormición de las semillas. Las mismas se incubaron a cinco temperaturas, se sometieron a escarificación mecánica y se lavaron con agua corriente para romper dormición. También se evaluó permeabilidad de coberturas seminales y presencia de inhibidores de la germinación solubles en agua. El rango óptimo de temperaturas estuvo entre 15-40°C para ambas especies. Se observó una respuesta positiva a todos los tratamientos pre-germinativos en *L. cuneifolia* (37-47%), mientras que *L. nitida* mostró un mayor porcentaje de germinación solo con escarificación (73%). Ambas especies presentan semillas permeables al agua, lo que descartó la existencia de una dormición física. El test de inhibidores fue positivo para *L. cuneifolia*, sugiriendo la presencia de una dormición química. Estos resultados son valiosos para la conservación y contribuyen a mejorar la producción de plantines en proyectos de restauración.

Palabras clave

inhibidores de la germinación • *Larrea cuneifolia* • *Larrea nitida* • temperatura óptima
• dormición física

INTRODUCTION

The genus *Larrea* is possibly one of the most widespread genera of desert shrubs on the American continent, including five species of xerophytic evergreen shrubs distributed in the different arid ecosystems (4, 27). One species (*Larrea tridentata*) inhabits in almost all the hot desert areas of North America (4). In South America, four other species (*Larrea ameghinoi* Speg., *Larrea cuneifolia* Cav., *Larrea divaricata* Cav. and *Larrea nitida* Cav.) are found along the Monte desert and the arid lands from Chaco and Patagonia, all of them being different biogeographic provinces of Argentina (14, 27). They are also found in arid areas of Chile, Bolivia, and Perú (14).

Germination studies on some *Larrea* species have demonstrated the presence

of a high percentage of dormant mature seeds (8, 30). Arid zones are characterized by a high percentage (> 80%) of shrub species that produce dormant mature seeds, mainly with physiological and physical dormancy (6, 26). According to Nikolaeva's (1977) simplified version, dormancy can be classified in physiological (factors within the embryo, inhibit germination), morphological (underdeveloped embryo), morphophysiological (combination of both), physical (impermeable seed coat), chemical (inhibitors in seed coverings), mechanical (seed coverings restrict radicle growth) and combinational. Dormancy itself can be eliminated by a particular factor, or by the combination of some of them (*e.g.*, light, temperature, and/or specific compounds).

Some authors found out that *L. divaricata* seeds present a type of physical dormancy caused by an impermeable seed-coat. They found that an efficient way of breaking it is through mechanical scarification with fine sandpaper (8, 30). On the other hand, *L. tridentata* seeds apparently present water-soluble germination inhibitors on the seed-coat (19), and removal of the coat or rinsing with running water have proved to enhance germination (from 27% in the control to 35-44%) (3, 19, 29, 33). However, neither one of these authors assessed the factors that produce the type of dormancy present in these seeds.

In some cases, dormancy classification is poorly done given that the factors that cause it are not well evaluated. It has been observed that mechanical or chemical scarification may also promote germination of seeds with physiological dormancy. In such instances, dormancy disruption by scarification appears to be related to the weakening of the embryo covering layer, allowing the radicle to emerge from it (5). Hence, it turns necessary to assess water uptake, comparing imbibition in scarified *versus* non-scarified seeds, in order to evaluate if seeds have water-impermeable seed-coats and therefore, make a correct dormancy classification. On the other hand, the presence of water-soluble germination inhibitors in the seed coat is not proved by any mean in several cases (5, 40). Thus, testing their presence by making a seed extract (31, 40) and using it as a substratum on germination assays, could help determine the existence of a chemical dormancy.

For this study, we selected two species of the genus *Larrea* which inhabits contrasting zones of the Monte Desert, and with unstudied germination biology.

On one hand, *L. cuneifolia*, which colonizes a widespread area, on the hotter and drier zones of the Monte Desert, and on the other hand, *L. nitida*, which is present in a more restricted range of colder zones, along watercourses, and linked with winter-type rains of Pacific origin (14, 38). Temperature is another critical abiotic factor that regulates seed germination and has important effects on dormancy and on the rate of germination of non-dormant seeds (7). Mean temperature expected for arid zone species is around 25°C (6, 34), and in general, germination temperature range is in accordance with the temperatures that are most favorable for seedling establishment and survival, but this could vary along their distribution area (12, 34, 39). According to the habitats where these two species grow, we could expect some differences in their germination temperature range and in the rate of the germination process.

The Monte biogeographic province is located in the western portion of Argentina, covering approximately 460.000 km². It is an arid region with water deficit almost all year and an average annual rainfall ranging from 30 to 350 mm. Its mean temperature ranges between 13 and 18°C (27, 37). These areas present moderate to severe degree of native ecosystem degradation (1), and restoration activities are challenging due to hard environmental conditions. *Larrea* species play a significant role in these ecosystems, besides being shelter and food for many small mammals and reptiles (10), they act as nurse plants for the establishment of other species (16, 36) and are keystone species of desert ecosystems (17, 23, 36). They consolidate the soil, given its wide and extensive radical system, forming extensive pure shrublands called "jarillales" (35). Currently, the lack of

information about seed germination and dormancy characteristics of these shrubs hampers to a large extent the production of saplings to restore degraded ecosystems in Argentina. Classifying seed dormancy of key species is a critical step in seedling production for restoration projects since it provides insights into suitable seed handling practices that promote germination (13, 20).

Considering their widespread distribution and their dominance in dry environments, to incorporate *Larrea* species into restoration programs of degraded areas, is a priority. Therefore, this study examined seed dormancy and germination requirements of two species of *Larrea* in order to efficiently produce a high amount of seedlings to restore arid regions of Argentina. Specifically, the hypotheses tested were

1- Seeds of *L. nitida* collected from cooler sites would have a greater germination rate at lower temperatures and a lower optimum temperature than *L. cuneifolia*.

2- According to the dormancy studies on other *Larrea* species, a physical dormancy imposed by their water-impermeable seed coat or a chemical dormancy due to the presence of water-soluble germination inhibitors in their seed coat may be present in these species. Based on this, we aim to determine the optimum germination temperatures of *L. cuneifolia* and *L. nitida* seeds, to analyze the response of two pre-germination treatments (scarification and rinsing with water), and to determine the type of seed dormancy these species have.

MATERIAL AND METHODS

Seed collection

Mature fruits of each species were collected from at least ten healthy adult shrubs: *L. cuneifolia* on March 2008 at the

experimental field of IADIZA (Instituto Argentino de Investigaciones de las Zonas Áridas), Parque General San Martín, Mendoza (32°53'43" S; 68°52'32" W) and *L. nitida* in December 2007 in San Antonio Oeste, Río Negro (41°19'38" S; 65°22'8" W). They were stored in paper bags and kept at room temperature (20-25°C) until experiments were performed in July 2008. The spherical hairy fruits are formed by five mericarps, each one typically containing one seed. To obtain the seeds, fruits of both species were rubbed between two rubber sheets, and only well-formed seeds were used in the assays. The number of empty mericarps, number of well-formed seeds and weight of 1000 seeds are shown in table 1 (measure of four replicates of 50 fruits and seeds, respectively). Half of the mericarps collected were empty (sterile) and a high number of well-formed seeds were observed (table 1).

Germination experiment

Germination assays were performed in incubators (Precision G. C. A. Corporation, Scientific Model 818), and in laboratory ovens (Dalvo, Model M. C/1/2) using 9 cm diameter Petri dishes prepared with cotton and a filter paper disk.

Table 1. Percentage of well-formed seeds, sterile fruits and weight of 1000 seeds of *L. cuneifolia* and *L. nitida* used in the assays (S.E. between parentheses).

Tabla 1. Porcentaje de semillas bien formadas, frutos estériles y peso de 1000 semillas de *L. cuneifolia* y *L. nitida* utilizadas en los ensayos (E.E. entre paréntesis).

Species	Sterile fruits (%)	Well-formed seeds (%)	Weight (g)
<i>L. cuneifolia</i>	55 (14.4)	96 (4.3)	4.09 (0.09)
<i>L. nitida</i>	49 (6.9)	95 (2)	3.32 (0.06)

This substratum was moistened to saturation with captan solution (commercial products) at 0.1% (w/v) to prevent fungal attack. The filter paper was remoistened with distilled water as necessary. In every test, four replicates of 25 seeds were randomly drawn from the total seed pool for each species and assigned to a treatment. Before germination treatments, seeds were surface sterilized in 15% sodium hypochlorite (36.8 g/l NaOCl) for 7 min, then rinsed three times with sterilized deionized water, and finally placed in Petri dishes. Experiments were conducted under constant (24 h) dark conditions.

Seeds were considered as germinated when the radicle reached more than 2 mm in length. The number of germinated seeds was registered daily and the germination capacity defined as the germination percentage cumulated over 15 days. Seeds viability was assessed with tetrazolium. In this test, imbibed seeds were cut in half and soaked in 0.5% tetrazolium chloride over 24 h at 30°C. Seeds with red embryos were considered viable and with white embryos, non-viable.

Temperatures

To determine the optimum germination temperatures of both species seeds were incubated in the dark, at constant temperatures of 10, 15, 25, 35 and 40°C. In preliminary assays, a high number of seeds under dormancy were observed, so a scarification treatment was done on both species to evaluate the effect of the different temperatures.

Besides the germination capacity, the Weighted Germination Percentage (WGP) was also measured in order to assess the rate of germination. This was calculated by giving maximum weight to the seeds that germinated first and progressively less

weight to those that germinated subsequently (32), for a time of fifteen days: $WGP = (15 \times n_1 + 14 \times n_2 + \dots + 1 \times n_{15}) / (15 \times N) \times 100$. Where: n_1, n_2, \dots, n_{15} = is the number of seeds germinated on 1st, 2nd, and subsequent days until the 15th day, respectively; 15, 14 ... and 1 are the weights given to the seeds germinated on 1st, 2nd, and subsequent days until the 15th day, respectively. N is the total number of seeds placed for germination.

Pre-germination treatments

For both species, we applied two pre-germination treatments following successful treatments applied to other species from the same genus: mechanical scarification and rinsing with running water. For mechanical scarification (MS) seeds were placed on fine sandpaper (N° 150) and gently rubbed one by one. And for rinsing, seeds were placed in small bags of fine gauze under running water during 24, 48 and 72 h (W24, W48, W72 respectively). After these treatments, seeds were placed in Petri dishes at 25°C.

Inhibitory activity test

To analyze if there were some soluble inhibitors on the seed coat, we performed a test using "alelí" seeds (*Mathiola incana*) (Sivouri EM 1964, not published). To extract the possible inhibitors 3 g of *Larrea* entire seeds were placed in glass beakers with 30 ml of distilled water (1:10 relation), one for each species. It was kept at 30°C in a laboratory oven for 72 h and periodically stirred with a glass rod. They were drained on a sieve over a funnel to collect the extract in a suitable container. This extract was used to moist the substratum (cotton and a paper filter disk) on the Petri dishes where alelí seeds were placed. As the osmotic potential of the extract was low (-0,02 MPa), it was

not necessary to make dilutions. A control with distilled water was also prepared. Both treatments were put under 25°C.

The inhibitor activity was obtained by assigning to the number of alelí seeds that germinated in the control (after 15 days) the relative value of 100, expressing on this basis the germination in the treatments with the extract.

Imbibition of scarified seeds

Imbibition of mechanically scarified seeds was compared with that of non-scarified seeds, to evaluate permeability of *L. cuneifolia* and *L. nitida* seeds after scarification treatments. For each species, three replicates of 100 scarified and non-scarified seeds were weighed and submerged in distilled water in Petri dishes at 20°C. After 72 h of imbibition seeds were removed, patted dry with a paper towel to absorb surface moisture and reweighed. Percent water absorption was determined gravimetrically. The amount of water taken up was determined by the increase in seed weight:

$$W(\%) = (w_i - w_d) / w_d * 100$$

where:

w_i and w_d = weights of imbibed and dry seeds, respectively.

Statistical analysis

Germination data (germination percentage and WGP) was subjected to a two-way analysis of variance (ANOVA) with species and temperature as factors. Germination (in pre-germination treatments), germination of Alelí seeds and water imbibition were subjected to a one-way ANOVA with treatments, incubation medium and scarification treatments as factors, respectively. Germination percentages were transformed

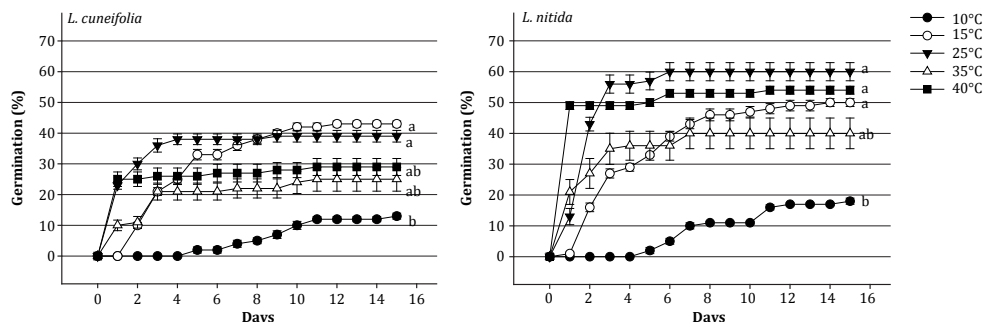
(arcsine square root) before the analysis in order to meet analysis assumptions. If significant differences were detected by ANOVA, Tukey's test was used for means comparisons. Statistical analysis was performed with Infostat 2013. Untransformed data appears in all figures and tables.

RESULTS

L. nitida seeds exhibited a higher germination capacity than did those of *L. cuneifolia*, and both species germinated in a wide range of temperatures (figure 1, page 241). In *L. nitida*, the average maximum germination capacity was 60% at 25°C, but it did not differ significantly from percentages reached at 15, 35, and 40°C ($F = 9.53$, $P = 0.0005$, figure 1, page 241). In *L. cuneifolia*, the maximum was 42% at 15°C and neither did it differ significantly from 25, 35 and 40 °C ($F = 6.13$, $P = 0.004$, figure 1, page 241).

At 10°C, seeds of both species attained lower germination percentages, and the process started later (5-6 days after the initiation of the assays) than over the other temperatures (1-2 days). Also, they reached two-thirds of the final germination percentage, over a wide range of temperatures (15-40°C), on the second day (figure 1, page 241). The interaction between species and temperatures was not significant ($F=1.15$; $P= 0.352$).

Weight germination percentage index (WGP) of both species at 10°C (6.1 and 9 for *L. cuneifolia* and *L. nitida*, respectively), was significantly lower than for the other temperatures ($F = 9.9$, $p = 0.0004$ for *L. cuneifolia* and $F = 14.8$, $p < 0,0001$ for *L. nitida*), so the germination process was slower. At the other temperatures WGP differences were not statistically significant.



Plots followed by the same letter are not significantly different at $p < 0.05$.

Las líneas seguidas por la misma letra no son significativamente diferentes para $p < 0,05$.

Figure 1. Time course of germination (mean \pm SE) for *L. cuneifolia* and *L. nitida* seeds at different temperatures.

Figura 1. Evolución temporal de la germinación (media \pm EE) de semillas de *L. cuneifolia* y *L. nitida* a diferentes temperaturas.

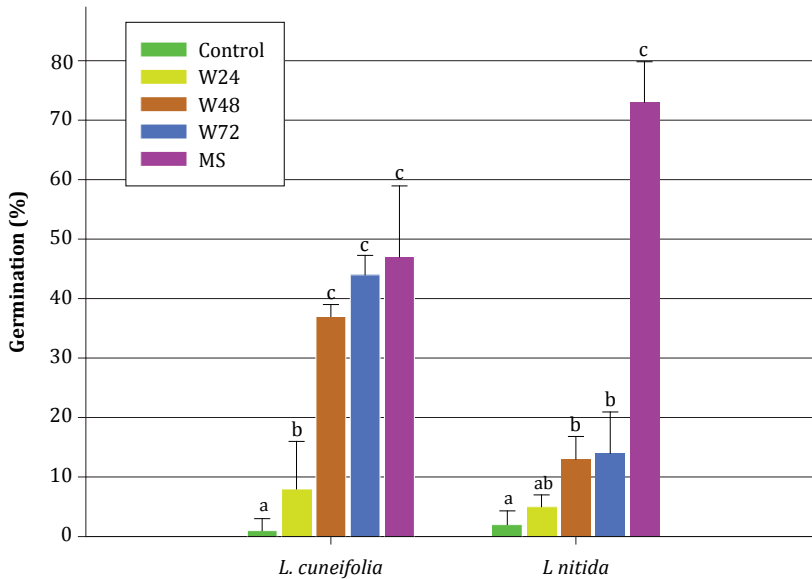
With respect to the pre-germination treatment, seeds of both species under control conditions germinated less than 2% (figure 2, page 242). Since seed viability was high (99% and 100% for *L. cuneifolia* and *L. nitida* respectively), almost all seeds were under dormancy. Dormancy release treatment by mechanical scarification enhanced final germination percentage of both species (figure 2, page 242), and higher values were obtained on *L. nitida* (figure 2, page 242). In *L. cuneifolia* seeds, mechanical scarification and rinsing for 48 and 72 h had the same effect on germination improvement (figure 2, page 242). Besides, with W48 and W72 germination processes was completed faster than with MS (1 day), since seeds were already imbibed in water and almost all of them (approximately 90%) germinated during the rinsing.

The inhibitory activity of *L. cuneifolia* seeds extract significantly reduced the germination percentage of alelí seeds with respect to the control (table 2, page 242). Whereas with *L. nitida* extract this difference

was not significant. Scarified seeds absorbed similar water percentages than non-scarified seeds in both species (table 3, page 243), and *L. nitida* absorbed less water than *L. cuneifolia*.

DISCUSSION

Since *Larrea* species are well adapted to arid environments, dormancy mechanisms allow a distribution of germination in time and space, ensuring that the environmental conditions are the most suitable for it to be completed (13, 15, 26). *L. cuneifolia* and *L. nitida* seeds presented dormancy mechanisms since almost all seeds did not germinate under control conditions, as mentioned for other species from the genus *Larrea* (3, 8, 30). Responses of both species to pre-germination treatments were different between them. Scarification promoted germination in both cases and rinsing with running water (W48 and W72) only promoted germination in *L. cuneifolia*.



Dissimilar letters for each species indicate significant differences ($p < 0.05$) among treatments.

Letras distintas para cada especie indican diferencias significativas ($p < 0,05$) entre los tratamientos.

Figure 2. Germination (mean \pm SE) for seeds of *L. cuneifolia* and *L. nitida* subjected to mechanical scarification (MS), rinsing with running water for 24, 48 and 72 h (W24, W48 and W72 respectively) and under control conditions (Control) at the end of the assay (15 days).

Figura 2. Germinación (media \pm EE) de semillas de *L. cuneifolia* y *L. nitida* sometidas a escarificación mecánica (MS), lavado con agua corriente durante 24, 48 y 72 h (W24, W48 y W72 respectivamente) y en condiciones control (Control) al final del ensayo (15 días).

Table 2. Mean germination of Alelí seeds (incubated at 25°C) under distilled water (control) and *L. cuneifolia* and *L. nitida* seeds extract at the end of the assay (15 days) and the ANOVA results.

Tabla 2. Germinación media de semillas de Alelí en agua destilada (control) y en extracto de semillas de *L. cuneifolia* y *L. nitida* al final del ensayo (15 días) y los resultados del ANOVA.

Species	Incubation Medium	Alelí seeds germination (%)	ANOVA results
<i>L. cuneifolia</i>	Control	81 (9.5) a	F=12.23 p=0.0027
	Seeds extract	39 (18.3) b	
<i>L. nitida</i>	Control	81 (9.5) a	F=3.94 p=0.0589
	Seeds extract	66 (9.5) a	

Dissimilar letters indicate significant differences ($p < 0.05$) among incubation medium within each species (SE between parentheses).

Letras distintas indican diferencias significativas ($p < 0,05$) entre los medios de incubación dentro de cada especie (EE entre paréntesis).

Table 3. Water imbibition (W) in scarified and non-scarified seeds of both species for 72 h, and their ANOVA results.**Tabla 3.** Absorción de agua (W %) de semillas escarificadas y no escarificadas de ambas especies durante 72 h, y los resultados del ANOVA correspondiente.

Species	Scarification treatment	W (%)	ANOVA results
<i>L. cuneifolia</i>	Non-scarified seeds	59 (5.61) a	F=1.38 p=0.3048
	Scarified seeds	64 (4.75) a	
<i>L. nitida</i>	Non-scarified seeds	34 (3.68) a	F=4.59 p=0.0989
	Scarified seeds	39 (1.41) a	

Dissimilar letters indicate significant differences ($p < 0.05$) among scarification treatments within each species (SE between parentheses).

Letras distintas indican diferencias significativas ($p < 0,05$) entre los tratamientos de escarificado dentro de cada especie (EE entre paréntesis).

Both species presented water-permeable seed coats, ruling out the occurrence of a physical dormancy. On the other hand, *L. cuneifolia* may have a chemical dormancy induced by the presence of water-soluble germination inhibitors. Besides, both species presented a wide range of germination temperatures, and there were no major differences between them in their optimum temperatures, as expected.

Temperature requirements of these two *Larrea* spp. were estimated through the different tested temperatures, where high percentages were observed around 25°C, a mean temperature expected for arid zones species (6, 34). The temperature at which seeds reach the higher germination percentage in the shortest time is called optimum germination temperature (12). In this case, both species presented a range of optimum temperatures between 15-40°C (27-42% for *L. cuneifolia* and 40-60% for *L. nitida*). Seed dispersal of native shrubs from the Monte Desert occurs mainly in summer, and seedlings emergence in the next spring-summer, when rainfall season starts (24, 25), which is in accordance with the temperatures

found for these *Larrea* species. Ostler *et al.* (2002), found in *L. tridentata* a greater germination percentage with soil temperatures of 23 °C during April, and its germination limits were between 10 and 40°C (3).

Since low germination percentages (2%) were observed in these species under control conditions, seeds were mechanically scarified before incubation at different temperatures. Consequently, the environmental range of conditions for the seeds to germinate becomes wider (2, 7) providing a possible explanation of the broad set of optimum temperatures observed in these species.

On the other hand, as *L. nitida* colonizes the colder parts of the Andes, in a more restricted range than *L. cuneifolia*, it was expected that a lower optimum temperature or a narrower temperature range than the ones found here, could be observed. These outcomes differ from the ones previously found on some *Prosopis* species that inhabit the Monte Desert, in which a higher germination capacity at low temperatures was observed in the species that present a more southerly distribution (12, 39).

A positive response to all dormancy-break treatments was observed in *L. cuneifolia*. *Larrea tridentata* from North America also presents similar responses to these treatments (3, 29, 33). The inhibitory test was positive since half of the alelí seeds did not germinate under *L. cuneifolia* seed extract, and the water uptake analysis showed that this species does not have water-impermeable seed coat. Therefore, it may be concluded that this species may have a type of chemical dormancy induced by water-soluble inhibitors present on the seed coat. The arid climates are characterized by scarce unpredictable rainfall, generally concentrated. After such rains, massive germination of plants may occur. This mechanism, observed in arid zones species, is the product of water-soluble germination inhibitors present in the seed coat. Gentle rains do not remove these inhibitors, so germination does not occur until the rains are more intense (22). Xylem-ring counts in *L. tridentata* seedlings showed that past germination and establishment occurred in response to heavy late-summer rainfall (9).

Despite these observations, chemical dormancy is not considered as such in many cases since there is not much evidence stating that seed dormancy, in nature, is regulated by the presence of inorganic compounds/ions in seed covering layers (5). Looking at the studies that test inhibitor activity of extracts (40), there is no possibility of knowing if the inhibitor came from the embryo and/or seed parts, nor if it would prevent embryo growth of non-dormant seeds of the species from which it was extracted. Besides, in many cases, species that present germination inhibitors also present a physiological dormancy. It has been observed that after this dormancy is broken, the embryo becomes insensitive to the inhibitors (6, 18). Therefore, the

chemical dormancy is being considered part of the physiological dormancy in the newest classification system (6). Seeds of *L. tridentata* under rinsing treatments showed similar germination percentages (3, 29, 33), but when leachates from their fruit coats were tested on their own seeds, it did not inhibit their germination as it did in other species (21).

On the other hand, *L. nitida* showed a higher response to mechanical scarification, but no significant differences were found in the water uptake measurements between scarified and non-scarified seeds. Therefore, this species does not present a physical dormancy caused by an impermeable seed coat, nor a chemical dormancy produced by germination inhibitors. The positive response to scarification could be related to a mechanical restriction of the seed coats, but this type of dormancy is actually under discussion since in many cases seeds also present physiological dormancy and after dormancy releases, the embryo has enough vigor to break seed covering layers (6). Another possibility may be a non-deep physiological dormancy, since as mentioned before, many species with this type of dormancy may increase germination with scarification, despite not having impermeable seed coats (5). Besides, physiological dormancy is one of the most common types in species from arid zones (6, 11). A study in *L. divaricata* detected higher germination percentages in seeds with mechanical scarification (88%), while soaking in gibberellic acid (GA_3) displayed similar results. The combination of both treatments resulted the most effective (30). This could also be the case of *L. nitida*. These species may have a physiological dormancy rather than a physical type. Therefore, it is necessary to continue studying in order to clearly define the type of dormancy of this species.

CONCLUSIONS

Larrea cuneifolia and *L. nitida* seeds presented dormancy mechanisms and scarification treatment promoted germination in both species, while rinsing with running water only promoted in *L. cuneifolia*. Both species presented water-permeable seed coats, ruling out the occurrence of a physical dormancy. On the other hand, *L. cuneifolia* may have a chemical dormancy induced by the presence of water-soluble germination inhibitors. Besides, both species presented a wide range of germination temperatures, and there were no major differences between them in their optimum temperatures, as expected.

The production of seedlings of *L. cuneifolia* and *L. nitida* for restoration programs implies a great effort and the knowledge of harvest times, quantity of sterile fruits, quality of seeds, dormancy level and pre-germination treatments. Our results allow us to better understand seed germination biology of this two *Larrea* species. Although we could not define the type of seed dormancy, and more research should be done in the future, this information is valuable and directly contributes to improving the efficiency of seed use and the production of a high amount of seedlings required for restoration projects.

REFERENCES

1. Abraham, E. M. 2002. Lucha contra la desertificación en las tierras secas de Argentina. El caso de Mendoza. In Fernández Cirelli, A.; Abraham, E. M. (Eds) El agua en Iberoamérica. De la escasez a la desertificación. Cooperación Iberoamericana CYTED Programa Iberoamericano de Ciencia y Tecnología para el desarrollo. Buenos Aires. p. 27-44.
2. Arana, M. V.; Gonzalez-Polo, M.; Martinez-Meier, A.; Gallo, L. A.; Benech-Arnold, R. L.; Sánchez, R. A.; Batlla, D. 2016. Seed dormancy responses to temperature relate to Nothofagus species distribution and determine temporal patterns of germination across altitudes in Patagonia. *New Phytologist*. 209(2): 507-520. Available in: <https://doi.org/10.1111/nph.13606>
3. Barbour, M. G. 1968. Germination requirements of the desert shrub *Larrea Divaricata*. *Ecology*. 49(5): 915-923. Available in: <https://doi.org/10.2307/1936543>
4. Barbour, M. G.; Diaz, D. V.; Breidenbach, R. W. 1974. Contributions to the biology of *Larrea* species. *Ecology*. 55: 1199-1215. Available in: <https://doi.org/10.2307/1935450>
5. Baskin, J. M.; Baskin, C. C. 2004. A classification system for seed dormancy. *Seed Science Research*. 14(1): 1-16. Available in: <https://doi.org/10.1079/SSR2003150>
6. Baskin, C. C.; Baskin, J. M. 2014. 2nd ed. *Seeds: Ecology, biogeography, and evolution of dormancy and germination*. Academic Press. San Diego. CA. USA. Available in: <https://doi.org/10.1017/CBO9781107415324.004>
7. Batlla, D.; Benech-Arnold, R. L. 2015. A framework for the interpretation of temperature effects on dormancy and germination in seed populations showing dormancy. *Seed Science Research*. 25(2): 147-158. Available in: <https://doi.org/10.1017/S0960258514000452>
8. Bonvissuto, G.; Busso, C. 2007. Germination of grasses and shrubs under various water stress and temperature conditions. *Phyton (B. Aires)*. 76: 119-131.
9. Boyd, R. S.; Brum, G. D. 1983. Postdispersal reproductive biology of a Mojave desert population of *Larrea tridentata* (Zygophyllaceae). *American Midland Naturalist*. 110(1): 25-36. Available in: <https://doi.org/10.2307/2425210>

10. Campos, C. M.; Borghi, C. E.; Giannoni, S. M.; Mangeaud, A.; Tognelli, M. F. 2006. Bark consumption of creosote bush (*Larrea cuneifolia*) by cuisas (*Microcavia australis*): Effect on branch survival and reproduction. *Ecología Austral*. 16(1): 1-6.
11. Commander, L. E.; Merritt, D. J.; Rokich, D. P.; Dixon, K. W. 2009. Seed biology of Australian arid zone species: Germination of 18 species used for rehabilitation. *Journal of Arid Environments*. 73(6-7): 617-625. Available in: <https://doi.org/10.1016/j.jaridenv.2009.01.007>
12. Cony, M. A.; Trione, S. O. 1996. Germination with respect to temperature of two Argentinian *Prosopis* species. *Journal of Arid Environments*. 33(2): 225-236. Available in: <https://doi.org/10.1006/jare.1996.0058>
13. Erickson, T. E.; Shackelford, N.; Dixon, K. W.; Turner, S. R.; Merritt, D. J. 2016. Overcoming physiological dormancy in seeds of *Triodia* (Poaceae) to improve restoration in the arid zone. *Restoration Ecology*. 24: S64-S76. Available in: <https://doi.org/10.1111/rec.12357>
14. Ezcurra, E.; Montana, C.; Arizaga, S. 1991. Architecture, light interception, and distribution of *larrea* species in the Monte Desert, Argentina. *Ecology*. 72(1): 23-34. Available in: <https://doi.org/10.2307/1938899>
15. Fitter, A. H.; Hay, R. K. 2002. *Environmental physiology of plants*. London. UK. Academic Press.
16. Fowler, H. G.; Whitford, W. G. 1996. Biogeography of desert annual plants: *Larrea tridentata* creosote bushes as islands. *Naturalia* (Sao Paulo). 103-111.
17. Gibson, A. C.; Sharifi, M. R.; Rundel, P. W. 2004. Resprout characteristics of creosote bush (*Larrea tridentata*) when subjected to repeated vehicle damage. *Journal of Arid Environments*. 57(4): 411-429. Available in: [https://doi.org/10.1016/S0140-1963\(03\)00120-4](https://doi.org/10.1016/S0140-1963(03)00120-4)
18. Kato, T.; Imai, T.; Kashimura, K.; Saito, N.; Masaya, K. 2003. Germination response in wheat grains to Dihydroactinidiolide, a germination inhibitor in wheat husks, and related compounds. *Journal of Agricultural and Food Chemistry*. 51(8): 2161-2167. Available in: <https://doi.org/10.1021/jf020998c>
19. Kay, B. L.; Ross, C. M.; Graves, W. L. 1977. Creosote bush. *Agronomy and range science dept. Mojave revegetation notes* (9).
20. Kildisheva, O. A.; Erickson, T. E.; Madsen, M. D.; Dixon, K. W.; Merritt, D. J. 2018. Seed germination and dormancy traits of forbs and shrubs important for restoration of North American dryland ecosystems. *Plant Biology*. Available in: <https://doi.org/10.1111/plb.12892>
21. Knipe, D.; Carlton, H. H. 1966. Germination and growth of some semidesert grassland species treated with aqueous extract from creosotebush. *Ecology*. 775-781. Available in: <https://doi.org/10.2307/1934264>
22. Lambers, H.; Chapin, F. S.; Pons, T. L. 2008. *Plant Physiological Ecology*. Springer-Verlag New York.
23. Laport, R. G.; Minckley, R. L.; Ramsey, J. 2012. Phylogeny and cytogeography of the North American creosote bush (*Larrea tridentata*, Zygophyllaceae). *Systematic Botany*. 37(1): 153-164. Available in: <https://doi.org/10.1600/036364412X616738>
24. Marone, L.; Rossi, B. E.; Horno, M. E. 1998. Timing and spatial patterning of seed dispersal and redistribution in a South American warm desert. *Plant Ecology*. 137(2): 143-150. Available in: <https://doi.org/10.1023/A:1009776601012>
25. Marone, L.; Horno, M. E.; Del Solar, R. G. 2000. Post-dispersal fate of seeds in the Monte desert of Argentina: Patterns of germination in successive wet and dry years. *Journal of Ecology*. 88(6): 940-949. Available in: <https://doi.org/10.1046/j.1365-2745.2000.00508.x>
26. Matilla, A. 2004. Ecofisiología de la germinación de semillas. In Reigosa, M. J.; Pedrol, N.; Sánchez, A. (Eds) *La Ecofisiología Vegetal. Una ciencia de síntesis*. Thomson-Paraninfo Editores. España. 901-922.
27. Morello, J. 1958. La provincia fitogeográfica del Monte. *Opera Lilloana*. 2: 5-115.
28. Nikolaeva, M. G. 1977. Factors controlling the seed dormancy pattern. In Khan, A. A. (Ed) *The physiology and biochemistry of seed dormancy and germination*. Amsterdam, North-Holland. 51-74.

29. Ostler, W. K.; Anderson, D. C.; Hansen, D. J. 2002. Pre-treating seed to enhance germination of desert shrubs. Bechtel Nevada Corporation. United States.
30. Palacio, L.; Baeza, M. C.; Cantero, J. J.; Cusidó, R.; Goleniowski, M. E. 2008. *In Vitro* Propagation of "Jarilla" (*Larrea divaricata* CAV.) and secondary metabolite production. *Biological & Pharmaceutical Bulletin*. 31(12): 2321-2325. Available in: <https://doi.org/10.1248/bpb.31.2321>
31. Qian, C. M.; Zhou, J.; Chen, L.; Su, Y. Y.; Dai, S.; Li, S. X. 2016. Bioassay of germination inhibitors in extracts of *Sapium sebiferum* seeds of different provenance. *The Journal of Horticultural Science and Biotechnology*. 91(4): 341-346. Available in: <https://doi.org/10.1080/14620316.2016.1160543>
32. Reddy, L. V.; Metzger, R. J.; Ching, T. M. 1985. Effect of temperature on seed dormancy of wheat. *Crop Science*. 25(3): 455-458. Available in: <https://doi.org/10.2135/cropsci1985.0011183X002500030007x>
33. Rivera, R. L.; Freeman, C. E. 1979. The effects of some alternating temperatures on germination of creosotebush (*Larrea tridentata* [D.C.] Con.: Zygophyllaceae). *Southwestern Naturalist*. 24: 711-714. Available in: <https://doi.org/10.2307/3670549>
34. Rodriguez Rivera, M. F.; Sosa, L. R., Fernandez, E. A.; Reale, M. I.; Villarreal, V. 2007. Efecto del estres hídrico a distintas temperaturas sobre la germinación de semillas de *Bulnesia retama* (Gill. ex. Hook.) Griseb. -zigofilaceas - En San Luis, Argentina. *Phyton*. 76: 5-17.
35. Roig, F. A. 1976. Las comunidades vegetales del piedemonte de la precordillera de Mendoza. *Ecosur*. 3: 1-45.
36. Rossi, B. E. 2004. Flora y vegetación de la Reserva de Biosfera de Ñacuñán después de 25 años de clausura. Heterogeneidad espacial a distintas escalas, PhD thesis. Universidad Nacional de Cuyo. Mendoza. Argentina.
37. Rundel, P. W.; Villagra, P. E.; Dillon, M. O.; Roig-Juñent, S.; Debandi, G. 2007. Arid and Semi-Arid Ecosystems. In Veblen T. T.; Young, K.; Orme, A. (Eds) *The Physical Geography of South America*. Oxford, Oxford University Press. 158-183.
38. Vidal Russell, R.; Tadey, M.; Calviño, C.; Souto, C. P. 2017. Citogeografía de *Larrea*, un género de distribución anfitropical en ambientes áridos de América del Norte y del Sur. In *Boletín de la Sociedad Argentina de Botánica*. 52 (Supl.). Proceedings of the XXXVI Jornadas Argentinas de Botánica. Mendoza. Argentina. 152.
39. Villagra, P.E. 1995. Temperature effects on germination of *Prosopis argentina* and *Prosopis alpataco* (Fabaceae Mimosoideae). *Seed Science and Technology*. 23(3): 639-646.
40. Wareing, P. F. 1965. Endogenous inhibitors in seed germination and dormancy. In Lang, A. (Ed) *Differenzierung und Entwicklung / Differentiation and Development*. Berlin. Springer. 2556-2571.

ACKNOWLEDGEMENTS

The authors would like to thank S. O. Trione for his expertise and assistance with the project, H. Debandi and G. Zalazar for assistance in the laboratory, and R. Pustavrh for improvements to the language in this manuscript.

The study was supported by Fundación Flora Nativa and Consejo Federal de Ciencia y Tecnología (PFIP 2007 N° 047).