

## PHYSICAL SEED DORMANCY IN NATIVE LEGUME SPECIES OF ARGENTINA

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**Summary:** Leguminosae is a family with high value of use for food, medicine, forage, ornamental and restoration ecology purposes. One obstacle for the use and management of many legume species is the presence of seeds with physical dormancy. Here, we evaluated the presence of physical dormancy in nine native species of Argentina and identified possible methods for breaking dormancy. *Caesalpinia gilliesii*, *Geoffroea decorticans*, and *Prosopis alata* have seeds with no physical dormancy, whereas *Crotalaria incana*, *C. pumila*, *C. stipularia*, *Desmanthus virgatus*, *Galactia texana*, and *Senna aphylla* have seeds with physical dormancy. The most effective methods for breaking physical dormancy were mechanical and wet heat (100°C) scarification for *Crotalaria* spp.; mechanical, acid (20 and 30 min) and wet heat scarification (80 and 100°C) for *D. virgatus*; mechanical scarification for *G. texana* and mechanical and all acid scarification treatments for *S. aphylla*. These results contribute to the knowledge of the germination biology for these species, and are of particular interest for their propagation in glasshouse and for restoration and conservation programs.

**Key words:** Leguminosae, physical dormancy, scarification, seed germination.

**Resumen:** Dormición física de las semillas de leguminosas nativas de Argentina. Leguminosae es una familia con un alto valor de uso como alimento, forraje, medicina y para fines ornamentales y de restauración ecológica. Uno de los principales problemas para el uso y manejo de muchas de estas especies es la presencia de semillas con dormición física. En el presente trabajo se evaluó la presencia de dormición física en nueve especies nativas de Argentina y los posibles métodos que permitan la salida de este estado. Las semillas de *Caesalpinia gilliesii*, *Geoffroea decorticans* y *Prosopis alata* presentaron semillas sin dormición, mientras que las semillas de *Crotalaria incana*, *C. pumila*, *C. stipularia*, *Desmanthus virgatus*, *Galactia texana* y *Senna aphylla* presentaron dormición física. Los métodos más eficientes para romper la dormición física de las semillas de las especies del género *Crotalaria* fueron la escarificación mecánica y el calor húmedo (100°C); la escarificación mecánica, la química (ácido sulfúrico: 20 y 30 minutos) y la escarificación con calor húmedo (80 y 100°C) para *D. virgatus*; la escarificación mecánica para *G. texana*; y la escarificación mecánica y la química (ácido sulfúrico: 10, 20 y 30 minutos) para *S. aphylla*. Estos resultados contribuyen al conocimiento de la biología de las semillas de estas especies y a su utilización en programas de propagación de plantas en invernaderos, restauración ecológica y conservación.

**Palabras clave:** Dormición física, escarificación, germinación, Leguminosae.

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## INTRODUCTION

The presence of one or more water-impermeable palisade cell layer(s) in seed or fruit coats inhibit water uptake and seed germination, and have been named as physical dormancy (PY). PY has been described in at least 18 Angiosperm families and several methods have been developed to break it and stimulate germination, such as mechanical and chemical scarification, dry or wet heat and dry storage (Baskin & Baskin, 2014). However, the presence of PY in a family does not mean that all species have this type of dormancy, and therefore its presence should be tested in each species individually. In addition, several reports have shown that even for a same species, responses may vary depending on provenance and/or season of collection (Burrows *et al.*, 2009; Piotta & Di Noi, 2003). The way to establish if seeds or fruits have water-impermeable coats is through imbibition studies (Baskin *et al.*, 2006).

Leguminosae is one of the families that are characterized by PY, although species without dormancy have been documented (Baskin & Baskin, 2014). This family has a high value of use, providing a highly nutritional food source for humans and animals. Other species are also used as source of timber, resins, insecticides, fibers, forage and medicines, as ornamental plants and for restoration ecology (Lewis *et al.*, 2005; Barboza *et al.*, 2009). Consequently, many populations are threatened by over-collecting. The knowledge of seed germination is fundamental for their management and use in plant propagation, restoration ecology and conservation programs. In this context, the specific objectives of our study were: (1) to evaluate the presence of PY in nine native legume species and (2) to determine the effects of wet heat, acid and mechanical scarification on dormancy breaking and seed germination of these species.

## MATERIALS AND METHODS

The studied species were *Caesalpinia gilliesii* (Wall. ex Hook.) D. Dietr., *Crotalaria incana* L., *Crotalaria pumila* Ortega, *Crotalaria stipularia* Desv., *Desmanthus virgatus* (L.) Willd., *Galactia texana* (Scheele) A. Gray, *Geoffroea decorticans*

(Gillies ex Hook. & Arn.) Burkart, *Prosopis alpataco* Phil., and *Senna aphylla* (Cav.) H. S. Irwin & Barneby. *Crotalaria* spp., *D. virgatus*, and *G. texana* are herbs or sub-shrubs species, *C. gilliesii*, *P. alpataco*, and *S. aphylla* are shrubs, and *G. decorticans* is a tree species. Taxonomy follows Zuloaga *et al.* (2008).

Mature fruits were collected throughout the period of natural dispersal of each species, from at least 10 individuals per species from different provinces of Argentina (Table 1). Seeds were separated from fruits, cleaned and stored at 15 °C and 15% relative humidity prior to the experiment ( $\leq 15$  d). To evaluate the presence of PY, an imbibition test was carried out. For each species, 25 seeds of intact and mechanically scarified (achieved by cutting the seed coat with a scalpel blade opposite to the micropyle) were weighed using a digital balance (0.0001g precision). Seeds were then sown in Petri dishes on the surface of 1% agar in water at 25°C under white light (8 h light/16 h dark). Seeds were removed from the dishes at 1-2 h intervals for the first 12 h and then every 24 h, blotted on filter paper to remove any surface moisture, and reweighed. The experiment was continued until the mechanically scarified seeds germinated ( $\geq 80\%$ ).

The effects of wet heat, acid and mechanical scarification on dormancy alleviation and seed germination, were established through the following treatments: 1) wet heat treatments (40°, 60°, 80°, and 100°C), in which seeds were immersed in water for two minutes; 2) acid treatments by immersing seeds in concentrated sulphuric acid for 10, 20 or 30 minutes; and 3) mechanical scarification. In the control, intact seeds with no pretreatment were used. Four replicates of 25 seeds for each treatment were then sown on the surface of 1% water agar in Petri dishes and germinated at 25°C (8-h light/16-h dark). The number of germinated seeds was recorded daily for 30 days, with germination defined as radicle emergence of at least 1.0 mm. Mean time to germination (MTG) in days was also calculated:

$$\Sigma (D n) / \Sigma n$$

where  $n$  was the number of seeds that germinated on day  $D$  and  $D$  was the number of days from the start of germination test.

Germination proportions were calculated and compared among treatments using ANOVA on

**Table 1.** Studied species including subfamily, provenance of samples and water uptake (% initial mass basis) of mechanically scarified and intact seeds. Values with the same letters are not significantly different at  $p > 0.05$ . Data are the mean  $\pm$  1 standard error.

Species	Subfamily	Province	Water uptake (% initial mass basis)	
			Mechanically scarified	Intact
<i>Caesalpinia gilliesii</i>	Caesalpinoideae	Buenos Aires	99.5 $\pm$ 6.2 <sup>a</sup>	96.9 $\pm$ 7.2 <sup>a</sup>
<i>Crotalaria incana</i>	Papilionoidea	Salta	193.4 $\pm$ 1.7 <sup>a</sup>	4.3 $\pm$ 2.6 <sup>b</sup>
<i>Crotalaria pumila</i>	Papilionoidea	Salta	168.3 $\pm$ 1.3 <sup>a</sup>	0.8 $\pm$ 0.1 <sup>b</sup>
<i>Crotalaria stipularia</i>	Papilionoidea	Salta	172.6 $\pm$ 1.3 <sup>a</sup>	1.4 $\pm$ 1.9 <sup>b</sup>
<i>Desmanthus virgatus</i>	Mimosoideae	Salta	152.2 $\pm$ 5.3 <sup>a</sup>	10.2 $\pm$ 2.1 <sup>b</sup>
<i>Galactia texana</i>	Papilionoidea	Santiago del Estero	95.6 $\pm$ 1.1 <sup>a</sup>	1.9 $\pm$ 0.2 <sup>b</sup>
<i>Geoffroea decorticans</i>	Papilionoidea	Buenos Aires	63.4 $\pm$ 25.8 <sup>a</sup>	56.0 $\pm$ 7.4 <sup>a</sup>
<i>Prosopis alata</i>	Mimosoideae	Neuquén	206.0 $\pm$ 3.6 <sup>a</sup>	227.6 $\pm$ 15.8 <sup>a</sup>
<i>Senna aphylla</i>	Caesalpinoideae	Neuquén	215.9 $\pm$ 8.9 <sup>a</sup>	20.2 $\pm$ 4.8 <sup>b</sup>

arcsine squared root transformed data. Means were compared by post hoc DGC's test (Di Rienzo *et al.*, 2002).

## RESULTS

After a week of imbibition, intact and mechanical scarified seeds of *Caesalpinia gilliesii*, *Geoffroea decorticans*, and *Prosopis alata* did not show differences in water uptake or seed germination ( $\geq 70\%$ ; Table 1), indicating that these species do not have PY. For *Crotalaria incana*, *C. pumila*, *C. stipularia*, *Desmanthus virgatus*, *Galactia texana*, and *Senna aphylla*, seeds mechanically scarified took up water and had an increase in mass of at least 95 %, whereas intact seeds only experienced a maximum weight gain of 20% (Table 1). Seed germination of these species was higher than 80% in mechanically scarified seeds and was completed during the first 3d, whereas germination in intact seeds was less than 10% and was delayed  $\geq 7$ d.

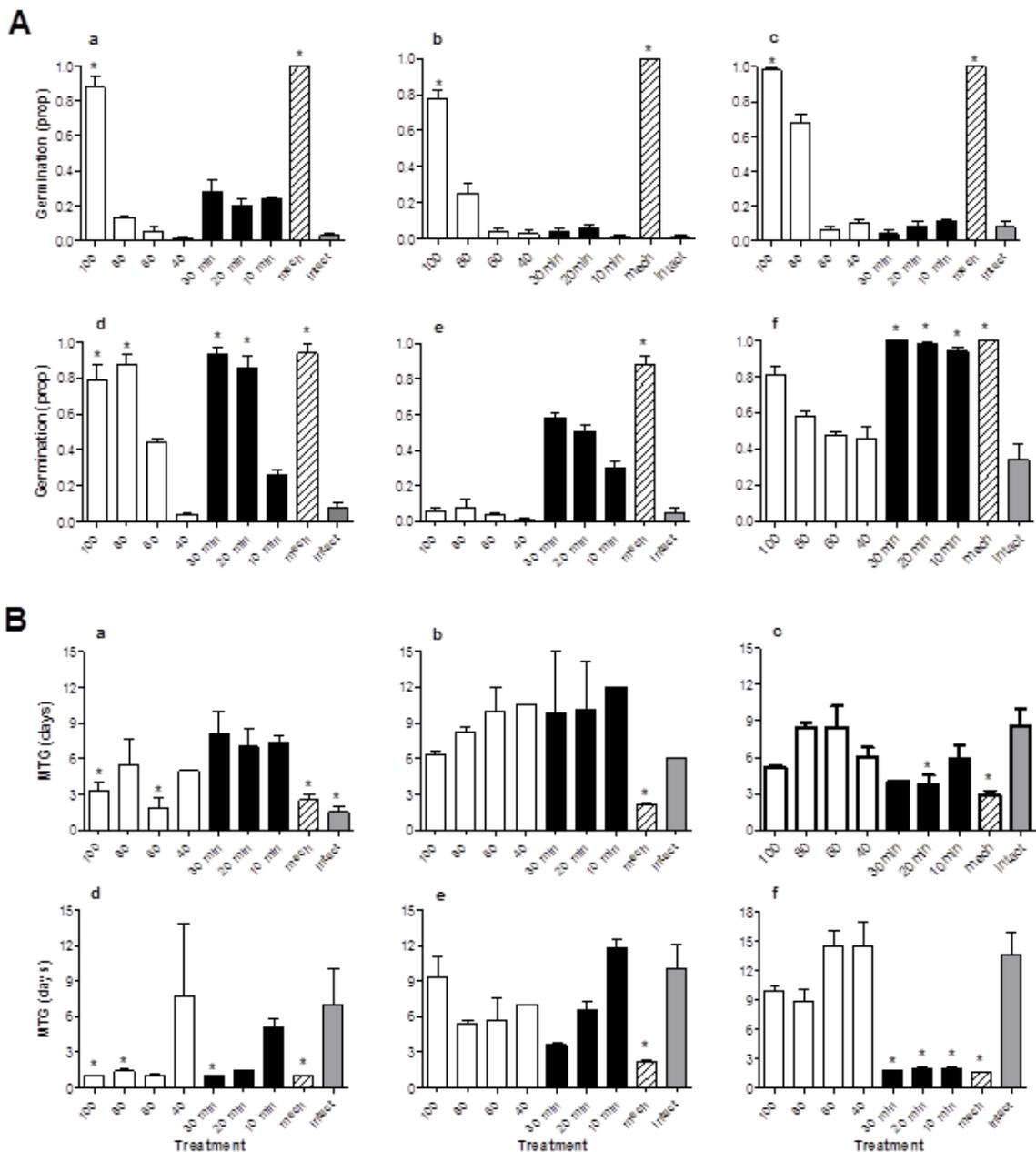
Germination and MTG were significantly different among dormancy-breaking treatments in species with PY (Fig. 1A, B). The most effective methods for breaking physical dormancy were mechanical and wet heat (100 °C) scarification for *Crotalaria* species (Fig. 1A a, b, c); mechanical, acid

(20-30 min) and wet heat scarification (80-100 °C) for *D. virgatus* (Fig. 1A d); mechanical scarification for *G. texana* (Fig. 1A e), and mechanical and all acid scarification treatments for *S. aphylla* (Fig. 1A f). For all species, germination started during the first two days after mechanical scarification. The lowest MTG was registered after wet heat (60-100 °C), mechanical scarification and in intact seeds for *C. incana*; after mechanical scarification for *C. pumila* and *G. texana*; in acid (20 min) and mechanical scarification for *C. stipularia*; in wet heat (80-100 °C), acid (30 min) and mechanical scarification for *D. virgatus* and after all acid treatment and mechanical scarification for *S. aphylla* (Fig. 1B a, b, c, d, e, f).

## DISCUSSION

The absence of physical dormancy in some legume species has been previously reported (Baskin & Baskin, 2014). Our results were consistent with those found by Figueroa & Jaksic (2004) for *G. decorticans* with other populations from Chile, and are the first to report the lack of PY dormancy for *C. gilliesii*.

Baskin *et al.* (2006) have stressed the importance of imbibition studies before concluding that



**Fig. 1.** (A) Germination proportion and (B) mean time to germination for each species under wet heat (white bars); acid scarification (black bars); mechanical (mech) scarification (hatched bars) and intact seeds (grey bars) treatments. Seeds were of *Crotalaria incana* (a), *C. pumila* (b), *C. stipularia* (c), *Desmanthus virgatus* (d), *Galactia texana* (e) and *Senna aphylla* (f). In each graph values with asterisk (\*) are significantly different at  $p < 0.05$ .

seeds are water-impermeable and implementing dormancy breaking experiments. For the remainder species evaluated in this study, previous works using seeds from other populations have concluded

that they have water-impermeable seed coats and therefore are physically dormant (Villagra, 1997; Carreras *et al.*, 2001; Hopkinson & English, 2004; Funes *et al.*, 2009; Reino *et al.*, 2011); however

critical experiments to prove their status were not performed. Therefore, and in order to unequivocally determine whether seeds have or have not PY dormancy, imbibition studies were performed in all species for the first time. Results show that *C. gilliesii*, *G. decorticans* and *P. alpataco* have seeds with no physical dormancy, whereas *C. incana*, *C. pumila*, *C. stipularia*, *D. virgatus*, *G. texana*, and *S. aphylla* have seeds with physical dormancy. These are in agreement with those previously reported, except for *P. alpataco*. Contrary to our results, Villagra (1997) has described this species as physically dormant, although no imbibition tests were conducted. These results highlight the importance of performing imbibition tests in order to accurately classify species regarding physical dormancy.

Under natural conditions, it has been suggested that exposition of seeds to high or fluctuating temperatures is the responsible for seed dormancy alleviation (Vazquez-Yanes & Orozco-Segovia, 1982; Van Assche *et al.*, 2003), whereas in laboratory conditions, the mechanical, thermal and chemical scarification, would be the most effective dormancy-breaking treatments (Baskin & Baskin, 2014). In this case, wet heat ( $\geq 80$  °C), acid and mechanical scarification were effective methods to break seed dormancy, depending on the species. Our results agree with those found for some of the species studied here (Godínez-Alvarez & Flores-Martínez, 1999; Carreras *et al.*, 2001; Lindig-Cisneros & Cabrera, 2004; Hopkinson & English, 2004; Funes *et al.*, 2009) and for other legume species (Ortega-Baes *et al.*, 2002; Galíndez *et al.* 2010; Baskin & Baskin, 2014). However, mechanical scarification may be very time-consuming, especially if large numbers of seeds are required (Baskin & Baskin, 2014). In this sense, it has been suggested that wet heat is a good option since it is a simple and reliable method, especially for plant propagation and restoration programs where large numbers of seeds are needed (Ortega-Baes *et al.*, 2002; Hopkinson & English, 2004).

The presence of seed dormancy interferes seriously with seed management and uses. The determination of presence and type of seed dormancy and the effectiveness of dormancy-breaking treatments, as has been done in this work, are fundamental for plant propagation

in greenhouse, seed conservation and also for understanding the environmental conditions that would favour the germination of these valuable legume species in their natural environments.

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