The role of seed germination in the invasion process of Honey locust (*Gleditsia triacanthos* L., Fabaceae): comparison with a native confamilial

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Abstract

Identifying plant traits that promote invasiveness has been a major goal in invasion ecology. Germination plays a central role in the life cycle of plants and therefore could be a key trait in determining species invasiveness. In this study, seed germination of two confamilial, co-occurring species that share ecological characteristics, the exotic invasive *Gleditsia triacanthos* L and the native *Acacia aroma* Gillies ex. Hook. & Arn., was compared. Seeds were obtained from individuals of three localities in the Chaco Serrano region of Córdoba, Argentina. Percent of seed germination and mean germination time were recorded in chemically and mechanically scarified seeds, and the former variable was also recorded in seeds subjected to: passage through the digestive tract of dispersers, fire simulations, fire simulation plus mechanical scarification, seed longevity, and dormancy break over time. In general, both species showed similar germination percentage. However, non-scarified seeds of the exotic species lost physical dormancy when subjected to experiments of dormancy break over time, whereas, the native species had shorter mean germination time. The greater percentage of seed germination over time of the exotic species than of the native one might be triggering the spread of the former, whereas the shorter mean germination time might be hindering its expansion to more arid regions. The study of different mechanisms for achieving seed germination, particularly in hard seed species, could provide important information on the expansion of invasive species as well as useful knowledge for their management.

Keywords: Acacia aroma, dormancy break, exotic invasive species, germination, *Gleditsia triacanthos*.

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Introduction

Exotic invasive species are considered a major threat to biodiversity, with significant impacts on ecosystem functions, such as fire regimes, hydrology, and nutrient cycling (Mack *et al*. 2000; Simberloff *et al*. 2005; Vilà *et al*. 2010). Indeed, their presence induces modifications, for example reduced water supply, that result in economic damage (Mack *et al*. 2000; Galatowitsch & Richardson 2005; Simberloff *et al*. 2005). Although extensive research on biological invasions has been developed (Quiroz *et al*. 2009; Gurevich *et al*. 2011), it is still necessary to increase knowledge on the ecology of invasive species for a better understanding of the invasive process (Gurvich *et al*.

2005b; van Kleunen *et al*. 2010; Lamarque *et al*. 2011). Accordingly, identifying plant traits that promote invasiveness has been a major goal, mainly through comparisons between native and exotic species (Daehler 2003; Pyšek & Richardson 2007; van Kleunen *et al*. 2010).

Strategies responsible for the invasiveness of exotic species include higher growth rate and fruit production than native species, or less prone to attack by natural enemies (Keane & Crawley 2002; Leishman *et al*. 2007; Mason *et al*. 2008; van Kleunen *et al*. 2010). Germination is a crucial step in the whole regenerative process and plays a central role in the life cycle of plants (Fenner & Thompson 2005); therefore, studying germination characteristics is important in order to gain knowledge about traits that might determine species invasiveness. Studies reviewed by Pyšek and Richardson (2007) indicated that

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exotic/exotic invasive species germinated earlier, better and under a wider range of conditions than native/exotic non-invasive species. On the other hand, a review by Daehler (2003) did not find significant differences in seed germination between native and exotic species. Hence, as with many other traits related to invasive exotic species to better understand the invasive process it is important to explore seed germination characteristics at the species level (Richardson & Pyšek 2006; Skálová *et al*. 2011).

Gleditsia triacanthos L. (Fabaceae) is a tree native to North America with a height of up to 15 m. It is considered one of the most successful woody leguminous invaders in different parts of the world (Richardson & Rejmánek 2011). In Argentina, it was introduced at the beginning of 19th century (Di-Iorio 2005) and is used as ornament, forest tree and windbreak (Lahitte & Hurrell 1998). Nowadays, this species is rapidly spreading, invading different ecoregions of the country, such as grasslands, subtropical montane forest ("yungas") and Chaco woodlands (Colombo Speroni & de Viana 2000; Grau & Aragón 2000; Marco & Páez 2000; Mazia *et al*. 2001; Ghersa *et al*. 2002; Zalba & Villamil 2002; Chaneton *et al*. 2004). In its native area *G. triacanthos* is considered a frequent invader of abandoned fields (Schnabel & Hamrick 1995), whereas in Argentina this species was suggested to be a gap colonizer (Mazia *et al*. 2001; Marco *et al*. 2002). In the Chaco Serrano Woodland of Córdoba, central Argentina, there are no con-generic species of *G. triacanthos*, so *Acacia aroma* Gillies ex. Hook. & Arn. was chosen as a native confamilial species for comparative purposes. *A. aroma*, of up to 5 m in height, is a common native leguminous species with South American distribution (Ebinger *et al*. 2000). Other characteristics shared by both species were taken as important criteria for their comparison: (i) a general overlap of reproductive phenology, that is, flowering in October to November and fruiting from December to September; and (ii) similar dispersal mechanisms and fruit morphology, that is, indehiscent legumes that fall by gravity from the plant and are then dispersed mainly by vertebrates (Ferreras & Galetto 2010). However, differences in seed germination were observed within the family and even within a single genus (Funes *et al*. 2009; Venier *et al*. 2012). These differences between *G. triacanthos* and a common native confamilial *A. aroma* may contribute to the understanding of the spread of the exotic species.

A previous study analyzing these species suggested that the exotic outperformed the native in some regenerative traits, for example, seed production, seed bank density, and germination of mechanically scarified seeds (Ferreras & Galetto 2010). However, there is a lack of studies involving a wider range of conditions that may either promote or hinder germination of *G. triacanthos* in relation to a common native confamilial. Hence, different germination experiments were performed considering possible factors

that may induce seed dormancy break. *Acacia aroma* and *G. triacanthos* seeds have a hard seed coat that prevents water uptake, that is, they have seeds with physical dormancy (Funes & Venier 2006; Tecco *et al*. 2012). In this type of seed, germination may be achieved by mechanical or chemical scarification. In the wild, seed passage through the digestive tract of dispersers is a common mechanism through which mechanical and/or chemical seed-coat abrasion may occur. *Acacia aroma* is mainly dispersed by foxes and *G. triacanthos* by cows (Colombo Speroni & De Viana 2000; Varela & Bucher 2006; Ferreras & Galetto 2010). For this reason, it is important to understand the effects of seed ingestion on seed germination (Traveset 1998; Traveset & Verdú 2002; Campos *et al*. 2008). Fire might also break seed dormancy, depending on fire temperature, velocity, and the depth of the seed within the soil profile (Baskin & Baskin 1998). Accordingly, the Chaco Serrano woodland has a long history of fire, mainly of anthropogenic origin (Miglietta 1994). In addition, in grassland ecosystems, increments in *G. triacanthos* seedling establishment were observed after a fire (Mazia *et al*. 2010). Finally, although the mechanisms by which seed permeability occurs are generally unknown, seeds of some species are known to lose physical dormancy after dry storage because their coats become permeable over time (Van Assche & Vandelook 2006). In addition, seed longevity may differ between native and exotic species and seeds with greater longevity may be able to survive until conditions for establishment are favorable (Vázquez-Yanes & Orozco-Segovia 1993; Schwienbacher *et al*. 2010). Accordingly, seeds of both species form a seed bank (Ferreras & Galetto 2010) and an increase in seed germination over time without scarification was observed in other Fabaceae species (Van Assche & Vandelook 2010). However, percentage of seed germination after different periods of storage is rarely evaluated in germination studies.

The aim of this study was to compare the germination capacity of an exotic invasive (*G. triacanthos*) and a native (*A. aroma*) species under a broad spectrum of germination experiments. We expect that this knowledge will contribute to a better understanding of the role of seed germination in the invasive process of the exotic species. A better germination capacity in the exotic species under any of the conditions evaluated suggests that the exotic species has a characteristic that promotes its invasive process in the Chaco Serrano Woodland of Córdoba. In addition, this information may be useful for the management of exotic species.

Materials and methods

Study sites

Seeds of *A. aroma* and *G. triacanthos* were collected from three sites where they co-occur in the Chaco Serrano

Woodland of central Argentina (Córdoba, Argentina): La Rancherita (31°45′41.2″S, 64°27′30.7″W), La Serranita (31°44′09″S, 64°26′43″W) and Villa Los Aromos (31°43′11″S, 64°26′23″W). Currently, these sites have low degrees of invasion by *G. triacanthos*, that is, there are few reproductive individuals with a density similar to or lower than that of *A. aroma*, depending on the site (average density in 20 m² was 1.13 ± 0.91 and 4.33 ± 1.87 individuals for *G. triacanthos* and *A. aroma*, respectively). However, in nearby areas (approximately 5 km away) the exotic species has become very abundant, forming almost monospecific woodlands, suggesting a possible rapid spread across the region.

Total annual precipitation in the study area is 734 mm, concentrated in summer, with a mean temperature of 24°C in summer and of 10°C in winter (De Fina 1992). The native woody vegetation includes *Lithraea molleoides*, *Zanthoxylum coco*, *Ruprechtia apetala, Celtis* spp., and other leguminous species, such as *Prosopis alba* and *Acacia caven*; some exotic species such as *Ulmus* spp. and *Ligustrum lucidum* are also present.

The area has a long history of fire mainly of anthropogenic origin, which has been increasingly used since the 16th century for land clearing in order to promote livestock raising and crop production (Albanesi *et al*. 2013). Nowadays, fires are a recurrent phenomenon in the area, that is, with a frequency of 2–15 years (Miglietta 1994; Gurvich *et al*. 2005a; Albanesi *et al*. 2013). They mainly occur from August to October, during the dry season, which coincides with the beginning of warm days (Miglietta 1994).

Germination experiments

Scarification treatments   First, three germination treatments were conducted in both species: (i) control—seeds obtained directly from the tree, without scarification; (ii) mechanical scarification—seeds were scarified with sandpaper; and (iii) chemical scarification—seeds were submerged in 98% sulphuric acid for 15 min and then washed with distilled water for 15 min to remove the residual acid from the seed surface. Treatments ii) and (iii) were intended to obtain relevant information about the degree of seed-cover permeability because seeds of both species exhibit physical dormancy (Funes & Venier 2006; Tecco *et al*. 2012). To determine whether seeds from control experiments and chemical treatments were dead or dormant, at the end of each experiment each seed was mechanically scarified. Seeds were again placed in the germination chamber until they could be classified as germinated or dead.

Seeds were collected from naturally fallen fruits of seven individuals of each species per study site (totalling 21 individuals per species) in 2008. Hence, each treatment consisted of 21 replicates of each species, each replicate containing 15 seeds. Seeds of the three treatments were placed in a germination chamber as described below.

Germination rate was determined for each treatment of the three germination experiments with the mean germination time (MGT) = $\Sigma(n \times d)/N$, where *n* is the number of seeds germinated between scoring intervals, *d* the incubation period in days at that time point, and *N* stands for the total number of seeds germinated in each treatment (Daws *et al*. 2002).

Passage through the digestive tract of dispersers   Feces from the main dispersers of each species (cattle and fox for *G. triacanthos* and *A. aroma*, respectively; Ferreras & Galetto 2010) were collected from La Rancherita study site during the fruit dispersal period of both species in 2008. Feces were collected only from one study site where they could be found in a large amount. From those feces, eight replicates consisting of 9–15 seeds were obtained, totalling 89 seeds of each species. Seeds were placed in a germination chamber as described below. After the end of the experiment each seed was mechanically scarified and placed again in the germination chamber until they could be classified as germinated or dead.

Fire simulation   Fire simulation experiments were performed in 2009. Seeds were collected from a minimum of five individuals per species, forming a pool of seeds from the three study sites. Seeds were divided into four treatments combining different temperatures and exposure periods: (i) 90°C, 1 min; (ii) 90°C, 5 min; (iii) 190°C, 1 min; (iv) 190°C, 5 min; and (v) without any heat treatment (control). The temperature ranges used were according to average minimal and maximal temperatures recorded for burned areas with comparable burning material (Jaureguiberry 2012) and were within the values suggested for this type of experiment in the literature (Teketay 1996; de Villalobos *et al*. 2002; Campbell & Clarke 2006; Figueroa *et al*. 2009). Seeds were placed on aluminium trays filled with a thin (about 2 cm) layer of soil from the study area. Seed sets were subjected to the simulated fire treatments (time/temperature combinations) in a dry oven, and then seeds were left to cool. Finally, seeds of each replicate were placed in individual Petri dishes. Three replicates of 15 seeds each were used for each treatment. A higher amount of seeds, and therefore of replicates, ideally should be used for this experiment, but the native species produced a low amount of seeds in that year. Seeds were placed in an incubation chamber, as described below, for 30 days to test the effects of fire simulation. After that period, non-germinated seeds were scarified mechanically and checked for 15 days to evaluate the combined effects of fire simulation plus mechanical scarification.

Seed longevity and dormancy break over time To evaluate seed longevity and dormancy break over a long period, seeds from naturally fallen fruits were collected from 17 individuals of each species during the dispersal period of both species in 2007 (from June to September). For this experiment, the seeds were collected from the La Rancherita study site only because the present study was part of a larger project for which sampling was restricted to that area in that year. A total of 13 germination experiments were conducted at different times between May 2008 and April 2011. Each experiment consisted in placing two replicates of each species with 10 non-scarified seeds each in a germination chamber (in August 2008 only one replicate of *G. triacanthos* was used because the other was attacked by fungi). Seed germination was checked for 15 days to evaluate dormancy break.

To evaluate seed viability, seeds that did not germinate in each of the 13 germination experiments previously described were mechanically scarified and placed in a germination chamber for another 15-day period. Total seed germination (scarified plus non-scarified seeds) was recorded for each period.

The time elapsed between experiments was variable, ranging from 8 days to 1 month (see Results, the periods between experiments were shorter as differences in seed germination became apparent). Seeds were stored in a refrigerator to avoid consumption by predators during the entire period of these germination experiments. The experiments were performed in a germination chamber as described below.

Incubation in a germination chamber The seeds used in the different germination treatments were placed on filter paper in 90-mm Petri dishes and moistened regularly. Petri dishes were placed in germination chambers at 25°C (12/12 h daily photoperiod, light density of about 40 μmol/m^2/s). The incubation temperature used was the optimal germination temperature for both species (Funes & Venier 2006; Tecco *et al*. 2012). Seeds were checked at 2–3-day intervals and germinated seeds were recorded and removed. Germination experiments were checked for 30 days (except for seed longevity and dormancy break over time). Protrusion of the radicle was used as the germination criterion.

Data analyses

For scarification treatments, differences in seed germination percentages and MGT between species were analyzed using an ANOVA block design, with "species" as a fixed factor and "study sites" as blocks (random factor). Intraspecific differences among treatments (mechanical, chemical, and non-scarified) were also analyzed with ANOVA block design, with "treatments" as fixed factors and "study sites" as blocks (random factor). Finally, an ANOVA block design was performed for each species, with "seed condition"—that is, dormant or dead—as fixed factor and "study sites" as blocks (random factor). *Arcsine$\sqrt{proportion}$* of seed germination or log$_{10}$MGT was used when parametric assumptions were not met. A Tukey test was performed for post-hoc comparisons among germination treatments. Passage through the digestive tract of dispersers was analyzed using an ANOVA test with "species" as a fixed factor. The ANOVA and ANOVA block designs were performed with SPSS 15 (SPSS Inc., Chicago, Illinois, USA).

For fire simulation, generalized linear models (GLMs) were performed with R 2.11.1(R Development Core Team 2010). Two models were constructed, one for "percentage of seed germination after fire simulation" and another one for "percentage of seed germination after fire simulation plus mechanical scarification". For both models, percentage of seed germination was analyzed as a function of "species," "treatments" (combination of heat and time), and the interaction between "treatments" and "species." Because overdispersion was detected, a quasibinomial error structure was used with a logit function. A backward elimination of the fixed effects was performed and the models were compared with an analysis of deviance until the best model was obtained. In all the analyses, a $P \leq 0.05$ was established as the criterion for rejecting the null hypothesis.

Results

Scarification treatments

No significant differences were found between the native and the exotic species in control, mechanical, or chemical seed germination treatments (Fig. 1; $F_{1,38} = 2.6$, $P = 0.12$; $F_{1,38} = 1.48$, $P = 0.23$; $F_{1,38} = 2.21$, $P = 0.15$ for non-scarified seeds, seeds after chemical scarification, and seeds after mechanical scarification, respectively). In both species, control treatment seeds that did not germinate were mostly dormant seeds (Table 1; all differences were statistically significant $P < 0.5$). In the chemical treatment, *G. triacanthos* exhibited a higher amount of dormant seeds, whereas *A. aroma* had a higher amount of dead seeds (Table 1; $F_{1,38} = 5.81$, $P = 0.02$; $F_{1,38} = 57.76$, $P = 3.84 \times 10^{-9}$ for *G. triacanthos* and *A.aroma*, respectively). In both species, mechanically scarified seeds presented a higher mean seed germination percentage than non-scarified or chemically scarified seeds (Fig. 1; $F_{2,60} = 50.74$, $P = 1.26 \times 10^{-13}$; $F_{2,60} = 100.36$; $P = 7.24 \times 10^{-20}$ for *A. aroma* and *G. triacanthos*, respectively). Mean germination time of mechanically and chemically scarified seeds of *G. triacanthos* was significantly greater than that of *A. aroma* (Table 2).

Passage through the digestive tract of dispersers

Percentage of seed germination after passage through the digestive tract of dispersers showed no significant differences between species ($F_{1, 14} = 0.41$, $P = 0.53$; Fig. 1). In both species most of the seeds that did not germinate were dormant seeds (Table 1). The values obtained in this experiment ($\bar{x} = 10.0 \pm 9.4\%$ and $\bar{x} = 7.1 \pm 8.8\%$ for
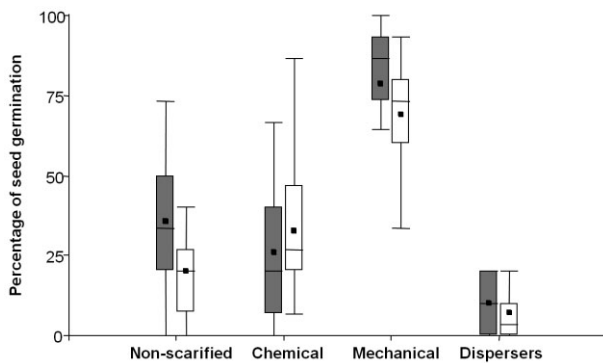

Fig. 1 Percentages of seed germination of the native *Acacia aroma* and the exotic invasive *Gleditsia triacanthos* under different germination treatments: non-scarified, chemically scarified seeds, mechanically scarified seeds and seeds after passage through the digestive tract of dispersers. Median and mean values are indicated by the perpendicular line within each box and the solid squares, respectively. No significant difference between species was found for any of the treatments. Among treatments, the mechanically scarified treatment showed significant differences with chemically scarified and non-scarified seeds. ■, *A. aroma*; □, *G. triacanthos*.

A. aroma and *G. triacanthos*, respectively) were lower than the ones obtained for control, that is, non-scarified seeds ($\bar{x} = 35.6 \pm 23.5$ and $\bar{x} = 20.2 \pm 12.3$ for *A. aroma* and *G. triacanthos*, respectively; Fig. 1).

Fire simulation

Both species had low germination percentages under different fire intensities (on average, less than 14%; Fig. 2a). These values were similar to the percentage exhibited by the control treatment. No statistically significant differences were found between each of the models and the null model (i.e., no differences were found between species and among treatments for fire simulation alone).

However, after mechanical scarification of those non-germinated seeds, differences in percentage of seed germination were observed between species and treatments (Fig. 2b). The final GLM for this experiment (i.e., fire simulation plus mechanical scarification) did not include the interaction between species and treatments because it did not present significant differences from a reduced model, that is, with only species and treatments (analysis of deviance, $F = 1.39$, $P = 0.27$). This reduced model showed differences with an even more reduced model (analysis of deviance, $F = 12.23$, $P = 0.002$). Therefore, the selected model (i.e., the most explicative and parsimonious one) was the one that included species and fire intensity treatments. In that model, significant differences between species were observed ($t = -3.38$, $P = 0.003$), with *A. aroma* showing lower germination values than *G. triacanthos*, particularly at 190°C, 1′ (Fig. 2b).

Table 1 Percentage ($\pm$ standard deviations) of dormant and dead seeds of *Acacia aroma* and *Gleditsia triacanthos* obtained after mechanical scarification from three treatments: control, chemical scarification and seeds passed through the digestive tract of dispersers; 100% represents the total amount of non-germinated seeds of each species in each experiment. Within species, for each treatment, all differences between dormant and dead seeds were statistically significant ($P < 0.5$). The percentage of seed germination is shown in Figure 1

	A. aroma		*G. triacanthos*	
Germination treatments	Dormant seeds (%)	Dead seeds (%)	Dormant seeds (%)	Dead seeds (%)
Non-scarified seeds (control)	66.5 ± 31.9	33.5 ± 31.9	78.4 ± 19.4	21.6 ± 19.4
Chemically scarified seeds	18.6 ± 26.1	81.4 ± 26.1	58.1 ± 21.2	43.1 ± 21.2
Passage through the digestive tract of dispersers	80.7 ± 16.1	19.3 ± 16.1	87 ± 9.3	13 ± 15.7

Table 2 Mean germination time (MGT) for different germination treatments in *Acacia aroma* and *Gleditsia triacanthos*. Mean $\pm$ standard deviations of MGT are shown and the number of replicates used is between parentheses (different replicates were considered for this test because those replicates with non-germinated seeds had to be excluded from the analysis)

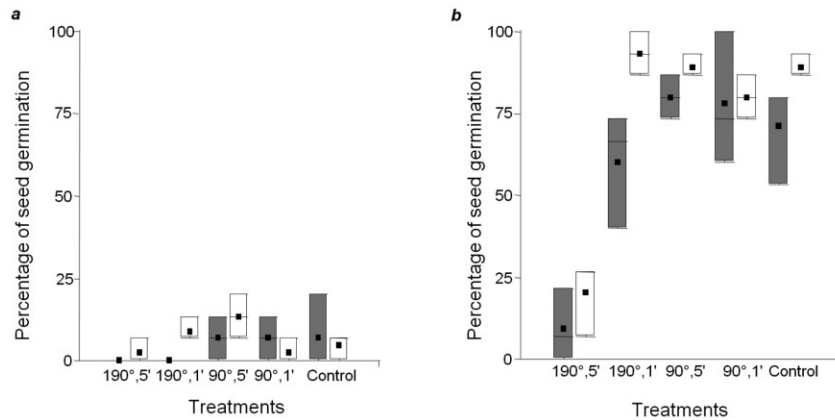|	Mean Germination Time (days)		
Germination treatments	*A. aroma*	*G. triacanthos*	Statistic
Non-scarified seeds (control)	16.9 ± 6.7 (n = 17)	17.7 ± 6.2 (n = 16)	$F_{1,29} = 0.13$, $P = 0.72$
Chemically scarified seeds	8.6 ± 3.1 (n = 16)	19.47 ± 7.3 (n = 15)	$F_{1,27} = 40.5$, $P = 8.18 \times 10^{-7}$
Mechanically scarified seeds	5.4 ± 1.1 (n = 20)	7.5 ± 1.1 (n = 21)	$F_{1,37} = 38.58$, $P = 3.25 \times 10^{-7}$

Fig. 2 Percentages of seed germination of the native *Acacia aroma* and the exotic invasive *Gleditsia triacanthos* (a) after different simulated fire treatments plus a control (no statistically significant differences were found among treatments or between species), (b) after mechanical scarification of the seeds used in (a). Significant differences were found among treatments, with 190°C, 5′ being different from the remaining treatments, and between species, with *G. triacanthos* having a higher germination percentage than *A. aroma*. Median and mean values are indicated by the perpendicular line within each box and the solid squares, respectively. ■, *A. aroma*; □, *G. triacanthos*

Table 3 Statistical analysis for differences among simulated fire treatments for seed germination percentages. This model (GLM) was constructed for the experiment in which seeds were exposed to simulated fire and then mechanically scarified

Treatments	190°C, 5′	190°C, 1′	90°C, 5′	90°C, 1′
Control	**t = 6.98**	t = 0.48	t = 0.69	t = 0.16
	P = 3.27 × 10⁻⁷	P = 0.63	P = 0.5	P = 0.87
190°C, 5′		**t = 6.7**	**t = 7.31**	**t = 6.89**
		P = 6.23 × 10⁻⁷	**P = 1.51 × 10⁻⁷**	**P = 4.03 × 10⁻⁷**
190°C, 1′			t = 1.16	t = 0.32
			P = 0.26	P = 0.75
90°C, 5′				t = −0.85
				P = 0.4
90°C, 1′				

Bold typeface indicates statically significant differences.

Differences among treatments were also detected, with the treatment of 190°C, 5′ showing significantly lower values of seed germination ($t > 6.7$, $P < 6.23 \times 10^{-7}$; Table 3) than the other treatments (Fig. 2b).

Seed longevity and dormancy break over time

Percentages of seed germination without scarification were low for both species at the beginning of the experiment; in *G. triacanthos*, however, a consistent increase in seed germination percentages without scarification was observed from June 2009 (Fig. 3a).

Seeds of both species germinated in all the experiments performed—that is, until April 2011—suggesting a viability of at least 3.5 years (Fig. 3b). In *G. triacanthos* total germination percentages were usually higher than 70%. In *A. aroma*, percentages were higher than 80% until May 2010 but since then a high variability in germination percentages was observed, ranging from 35 to 75% (Fig. 3b).

Discussion

Acacia aroma and *G. triacanthos*, in general, did not show significant differences in germination characteristics. However, these species showed some differences in seed germination that may be involved in the exotic species expansion.

On the one hand, the species did not show significant differences in germination percentage in any of the treatments (non-scarified (control), mechanically or chemically scarified seeds). These results suggest that both species tend to have similar seed-cover permeability. However, some differences were observed in the amount of dead seeds after the chemical scarification treatment, suggesting that the native species may have suffered more severe damage than the exotic one. This could have implications in the passage through the digestive tract of dispersers other than the ones evaluated in this study. As in many other Fabaceae, both species showed highest germination percentages under mechanical scarification (Baskin &
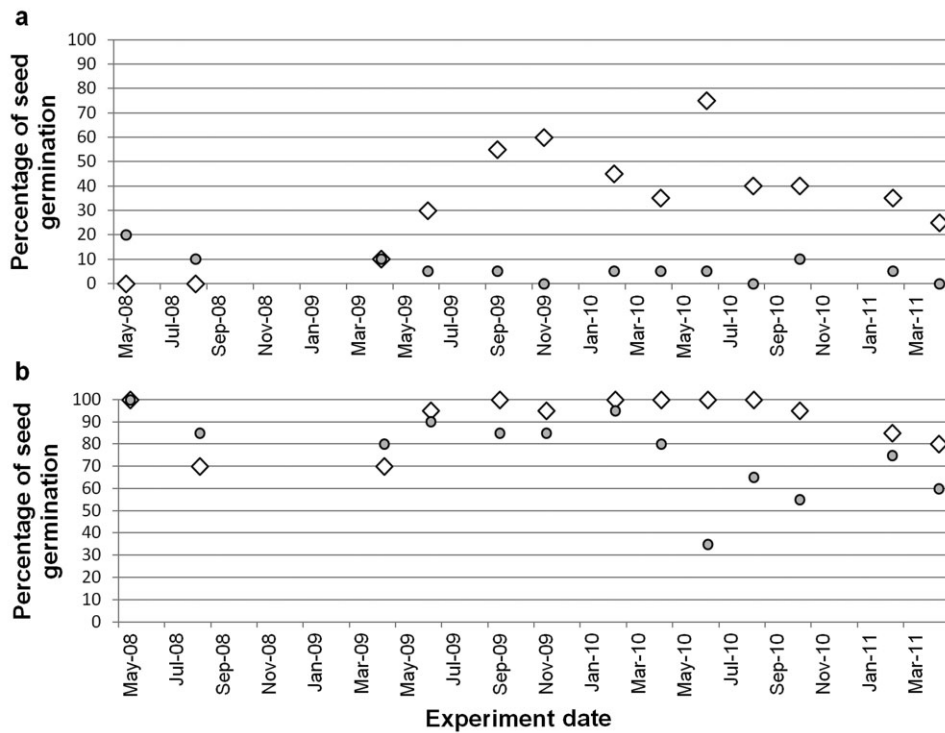
Fig. 3 Percentages of seed germination over 3 years (seeds were collected in 2007) for the native *Acacia aroma* and the exotic invasive *Gleditsia triacanthos*. Each data point represents the average value for the two replicates performed at the corresponding experimental date. (a) Percentage of seed germination without mechanical scarification. (b) Total percentage of seed germination (scarified plus non-scarified seeds). ◇, *G. triacanthos*; ◉, *A. aroma*.

Baskin 1998; Ortega Baes *et al*. 2002) and those percentages were similar to values reported for other Fabaceae of the region (Funes & Venier 2006; Funes *et al*. 2009). Different mechanisms of physical dormancy break in the field have been proposed, such as abrasion by soil particles (Baskin & Baskin 1998), wet heat (Hu *et al*. 2009), temperature fluctuations (Taylor 2005; Ventura de Souza *et al*. 2012), or partial damage produced by seed predators at early developmental stages (Vallejo-Marín *et al*. 2006). In the study area, many of those factors may be involved in mechanical scarification of the seeds, such as wet heat in summer due to high temperatures and precipitation, or wide temperature fluctuations in autumn or spring. In addition, signs of predator-mediated scarification had been observed in other species of *Gleditsia* (Takakura 2002) and in *G. triacanthos* in the study area (Ferreras & Galetto 2010). Hence, studies focusing on the mechanisms promoting mechanical scarification in the field would be needed. Finally, in a previous regeneration study performed with both species in 2007, which included germination of mechanically scarified and non-scarified seeds, a higher germination percentage was found in the scarified seeds of *G. triacanthos* than in those of *A. aroma* (Ferreras & Galetto 2010). Those results may be attributed to varia-

tions in seed properties between growing seasons (Fenner 1991; Fenner & Thompson 2005). In addition to those variations, percentage of seed germination may vary among individuals within each species. This pattern has been observed in individuals of other Fabaceae invasive species (*Robinia pseudoacacia*) (Masaka & Yamada 2009). In this study the general pattern between species seems to be clear (Fig. 1) and the variability among individuals would not be as important as in *R. pseudoacacia*. However, this source of variation deserves new studies.

On the other hand, *A. aroma* seeds had shorter germination times than *G. triacanthos*, which were statistically significant at least for chemically and mechanically scarified seeds. This trait may be considered an advantage for the native species over the exotic one, because native seeds might use the environmental resources available for establishment earlier than exotic seeds. However, this could be more important in areas with high water limitation, which is not the case of the study area, except at the beginning of the rainy season when rain events are sporadic. Accordingly, the longer germination time in *G. triacanthos* might hinder its expansion to more arid regions and agrees with the pattern currently observed in Argentina, where the species is generally absent in arid areas (de Viana &

Colombo Speroni 2000; Grau & Aragón 2000; Marco & Páez 2000; Mazia *et al.* 2001; Ghersa *et al.* 2002; Zalba & Villamil 2002; Chaneton *et al.* 2004).

Seed dispersal by animals, one of the many ways in which seed dormancy can be overcome in nature, did not seem to increase seed germination in either species in the present study. In addition, the percentages obtained were even lower than values recorded in the control treatment. Similar results were reported in previous studies that included germination of these species after seed passage through the digestive tract of dispersers (Colombo Speroni & De Viana 2000; Varela & Bucher 2006, for *G. triacanthos* and *A. aroma*). As these results were obtained from feces of just one study site, further studies including more populations would be needed. Although seed passage through these dispersers' digestive tract does not produce seed scarification, it can contribute to other processes that could be important for species establishment, such as the removal of the fruit cover—both species have indehiscent fruits—the movement of seeds to new places for establishment, and/or the provision of a nutrient source—that is, feces—where seedlings could grow (Traveset 1998; Traveset *et al.* 2008). Venier *et al.* (2011) reported enhancement of *A. aroma* seed germination after simulated cattle ingestion. In our study sites, cattle feces contained numerous seeds of *G. triacanthos* (on average 409 ± 354 seeds per fecal unit, averaged performed from just four of the feces used in the study) and a low number of seeds of *A. aroma* were found (maximum four seeds per feces). Therefore, before *G. triacanthos* introduction to our study area, cattle may have consumed more *A. aroma* seeds than at present, because it is one of the few species that provides fruits during winter. Hence, cattle might have represented an important contribution to *A. aroma* establishment. Although cattle have a long history in the area (Renison *et al.* 2010), they are also exotic and therefore their introduction may have further facilitated *G. triacanthos* expansion. This phenomenon has been observed in other exotic species and was described by the "Invasional meltdown hypothesis" (Simberloff & Von Holle 1999; Simberloff 2006).

Fire is another natural agent that might contribute to seed dormancy break (Baskin & Baskin 1998) and has been associated with increments in exotic species abundances (D'Antonio 2000; Brooks *et al.* 2004; Keeley *et al.* 2005; Mazia *et al.* 2010). In the area, fires had a frequency of 2–15 years (Miglietta 1994; Gurvich *et al.* 2005a). As with the previous treatments, these species did not show an increase in seed germination after the different fire intensity treatments, suggesting that the simulated fire intensities did not contribute to seed dormancy break in these species. However, the seeds that were also mechanically scarified showed significant differences among treatments and between species in the final model. Within treatments, the decrease in seed germination at the highest temperature/time treatment—that is, 190°C, 5′—suggested that this fire intensity affected seed viability of both species (Fig. 2b). Between species, *A. aroma* had a lower percentage of seed germination, particularly at 190°C, 1′ (Fig. 2b). However, studies with a broader range of fire intensities are needed in order to provide more information about the exact array of intensities at which these species may differentiate. In addition to the direct effects of fire on seeds, fire could relieve the species from their competitors and change nutrient availability, which could have additional effects on species establishment, particularly promoting exotic species invasion (Chaneton *et al.* 2004; Mazia *et al.* 2010).

Germination percentage of stored seeds of the exotic species *G. triacanthos* experienced an increase without scarification about 20 months after fruits had naturally fallen from the tree (Fig. 3a). Even though this phenomenon has rarely been studied, it has been observed in other Fabaceae and Geraniaceae species (Van Assche & Vandelook 2006, 2010). Therefore, through this capacity of breaking seed dormancy over time, seeds of *G. triacanthos* might germinate years later. This trait seems to be a key factor in determining differences in seed germination strategies between species and could be important for the spread of exotic species. The mechanism through which seed germination is achieved in this kind of experiments—that is, dormancy break over time—is unknown (Van Assche & Vandelook 2006); however, as those seeds were stored in a refrigerator, exposure to low temperature could be a mechanism that facilitates seed germination. In the wild, even though both species form a seed bank, significant differences in the amount of seeds in the soil between them were observed, being higher in *G. triacanthos* (Ferreras & Galetto 2010; Ferreras *et al.* data not shown). Considering, also, the seasonal climate at the study site and that the seeds would remain at least for one winter in the soil, germination can occur after many months in *G. triacanthos*. Nevertheless, new experiments considering germination over time with *in situ* assays are needed for a better understanding of the ecological consequences. In addition, as this experiment was restricted to one population of both species, changes in seed properties among populations could have modified these results. Finally, the chance of seeds to germinate almost 3 years after fruits have naturally fallen from the tree increases the opportunity for establishment under favorable conditions in both species. This must be particularly important in the exotic species because *G. triacanthos* showed a high density of seeds in the seed bank (Ferreras & Galetto 2010), which could remain viable until conditions for establishment are favorable.

Conclusions

Both species were similar in most of the characteristics studied, as suggested by Daehler (2003) for other native–exotic pairs. However, differences in seed germination over time could be a key process involved in *G. triacanthos* expansion. These differences might be triggering the spread of the exotic species in this area and are in agreement with the results reported by Pyšek and Richardson (2007) for other native–exotic comparisons. Finally, other differences between species (i.e., MGT) may favor the regeneration process in the native *A. aroma* and could be hindering the invasion of the exotic species to more arid areas of the country. Mechanisms that trigger germination are complex and information such as the mechanisms necessary for mechanical scarification or germination variability among individuals might be important for a better understanding of an invasion process. In addition, other vegetative and regenerative strategies might be studied for a better understanding of the invasive process of *G. triacanthos* (Tecco *et al.* 2012).

Acknowledgments

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