



Pre-dispersal seed loss in two *Prosopis* species (Fabacea: Mimosoidea) from the Monte Desert, Argentina

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ABSTRACT. Pre-dispersal filters imposed on the seed stage can alter the likelihood of seed dispersal. We evaluate pre-dispersal seed loss due to predation by insects and abortion in *Prosopis flexuosa* and *P. chilensis*. This study was conducted in two protected areas in the Monte Desert. We collected *P. flexuosa* and *P. chilensis* fruits from different trees, from two plots and two years. Samples were maintained for 50 days in translucent PVC bottles stored in a laboratory under stable temperature (25 °C) and natural photoperiod, awaiting the emergence of insects. Then we opened the fruits and individually examined all seeds to determine their condition. We found that total pre-dispersal seed loss was 32% in *P. flexuosa* and 21% in *P. chilensis*. Seed predation by insects was the major source of pre-dispersal seed loss (19% in *P. flexuosa* and 14% in *P. chilensis*). The main seed predator was the apionid weevil (Brentidae: Apioninae) in *P. flexuosa*, and bruchid beetles (Chrysomelidae: Bruchinae) in *P. chilensis*. Some bruchid beetles prey upon seeds, completing their life cycle, whereas others remain inside seeds (41% in *P. flexuosa* and 49% in *P. chilensis*, of total seed damaged by bruchid beetles). Seed abortion was another important source of seed loss, especially for *P. flexuosa*, but its cause still remains unknown. We show and discuss the extent of a proposed methodology to account for pre-dispersal seed predation that includes the immature stages of non-emergent bruchid. Pre-dispersal seed loss by insects and abortion represent an ecological filter that limits the amount of seeds available for dispersal and establishment of these species. Understanding seed loss process may contribute to know and predict *Prosopis* population dynamics, revealing the natural regeneration mechanisms to forest recovery.

[Keywords: abortion, insects, predation]

RESUMEN. Pérdida de semillas en fase de pre-dispersión en dos especies de *Prosopis* (Fabacea: Mimosoidea) del Desierto del Monte, Argentina. Los filtros impuestos sobre las semillas en la fase pre-dispersiva pueden alterar su probabilidad de ser dispersadas. Evaluamos la pérdida de semillas debido a la depredación por insectos y abortación durante la etapa predispersiva en dos especies: *Prosopis flexuosa* y *P. chilensis*. El estudio se realizó en dos áreas protegidas del Desierto del Monte. Colectamos frutos de ambas especies de *Prosopis* a partir de individuos adultos de dos parcelas y en dos años. Las muestras se colocaron en frascos de PVC transparentes almacenados en laboratorio a una temperatura estable (25 °C) y fotoperíodo natural durante 50 días, para determinar la emergencia de los insectos. Luego se abrieron los frutos y se examinó cada semilla para registrar su condición. La pérdida total de semillas fue del 32% en *P. flexuosa* y del 21% en *P. chilensis*. La depredación de semillas por insectos fue la fuente principal de pérdida de semillas (19% en *P. flexuosa* y del 14% en *P. chilensis*). Los depredadores más importantes fueron *Apion* sp. (Brentidae: Apioninae) en *P. flexuosa*, y brúquidos de distintas especies (Chrysomelidae: Bruchinae) en *P. chilensis*. Algunos brúquidos depredan la semilla y emergen por un pequeño orificio al completar su ciclo, mientras que otros permanecen en el interior de la semilla (41% en *P. flexuosa* y del 49% en *P. chilensis* del total de semillas depredadas por brúquidos). La abortación fue la segunda fuente de pérdida predispersiva de semillas, en especial para *P. flexuosa*, aunque aún se desconocen sus causas. Mostramos y discutimos el alcance de una metodología para la estimación de depredación pre-dispersión, que considera la inclusión de los estadios inmaduros de brúquidos que no emergen de la semilla. La pérdida de semillas pre-dispersión por insectos y abortación, representan un filtro ecológico que podría limitar la cantidad de semillas disponibles para la dispersión y posterior establecimiento de estas especies. Comprender el proceso de pérdida de semillas puede contribuir a conocer y predecir la dinámica poblacional de *Prosopis*, revelando los mecanismos naturales de regeneración para la recuperación de los bosques.

[Palabras clave: depredación, insectos, abortación]

INTRODUCTION

Biotic and abiotic pre-dispersal filters imposed on the seed stage can alter the likelihood of seed dispersal (Janzen 1971), affecting the probability of transition to the following stages (seedling, juvenile and adult). Pre-dispersal filters can be represented by the species' intrinsic factors such as patterns of resource allocation, floral biology, compatibility (Stephenson 1981), abiotic factors such as light, temperature, resource availability (Stephenson 1981; Verdú and García-Fayos 1998) and biological interactions (e.g., competition, pollination, and predation) (Jordano et al. 2004).

The main cause of pre-dispersal loss of reproductive structures is abortion, which can significantly limit seed production (Stephenson 1981; Sutherland 1986). Seed abortion can have an ecological or evolutionary origin (Stephenson 1981). For example, abortion can be an adaptive strategy if a high proportion of flowers that later abort increases the proportion of both pollen donation and fertilization by attracting more pollinators (Ayre and Whelan 1989). Plant-animal interactions can also result in seed abortion because pollination failure (Bawa and Webb 1984; Wilcock and Neiland 2002) and predation of flowers and unripe fruits by insects (Pettersson 1991) limit the proportion of fertilized flowers, which become healthy fruits and viable seeds (Stephenson 1981; Mahoro 2003). Pre-dispersal seed predation by insects is another cause of pre-dispersal seed loss which affects plant population dynamics (Louda 1989; Crawley 2000) and have the potential to negatively influence seed dispersal mechanisms (Jordano 1987; Sallabanks and Courtney 1992; Bas et al. 2005). This process may bring about the destruction of a great proportion of the seeds produced, limiting the number of seeds to be dispersed (Janzen 1971; Louda 1983; Jordano et al. 2004), local recruitment (Louda 1982), establishment of new individuals (Louda 1983; Louda 1990; Wang and Smith 2002) and population growth (Kolb et al. 2007).

Pre-dispersal seed predators are represented by a wide variety of animals, but many of them are inconspicuous specialized insects that attack a particular plant species or a few closely related species (Hulme 2002). Seed beetles, also named bruchid beetles (Chrysomelidae: Bruchidae), are especially common predators and parasites of legume seeds in drylands and tropical environments, and many are highly host-specific (Ramírez and Traveset 2010).

In many legume species, including *Prosopis*, bruchid beetles can destroy most of the seeds produced (Solbrig and Cantino 1976; Kingsolver et al. 1977; Zimmermann 1991; Kistler 1995), being in some cases important demographic hurdles (e.g., Midgley and Bond 2001). Bruchid females oviposit on or inside fruits and larvae generally prey upon a single seed (Southgate 1979). Then they pupate after several larval stages, which in most cases kill the embryo, or consume much of the endosperm, preventing germination (Camargo-Ricalde et al. 2004; El Atta 2000; Tomaz et al. 2007). In some cases, seed-beetle larvae may attack a majority of seeds in the local plant population, but infection rates of some hosts can be chronically low (Miller 1994; Takakura 2002).

Seed predation rates by bruchid beetles show a high variability among host species and regions, ranging from 3% to 90% on different populations of *Prosopis* species (Table 1). The intensity of seed predation by bruchids depends of both ecological factors (e.g., larval competition, parasitoids, heat and desiccation of eggs; Traveset 1991) and intrinsic traits of the species, such as multivoltine life cycle, amount of eggs laid per female (Southgate 1979), habits and preferences of seed predator species (Jansen 1980; Johnson and Romero 2004; Szentesi et al. 2006). Bruchid beetles may oviposit differentially according to both the stage of fruit ripening and the location of fruits in relation to their parent tree. For example, some species prey on seeds throughout the fruit ripening process (Kingsolver et al. 1977), while others prefer exclusively immature (Kingsolver et al. 1977; Impson et al. 1999) or mature seeds (Johnson and Romero 2004). Other bruchid species develop and complete their life cycle inside mature seeds, but only in fruits still attached to the parent plant, while others prefer to prey on seeds when fruits are already on the ground (Miller 1996; Johnson and Romero 2004). When fruits are not removed by endozoochorous herbivores, some multivoltine bruchids species can have several generations re-infesting healthy seeds in long term available pods on the ground (Ortega Baes et al. 2001).

Most *Prosopis* species may produce abundant flowers during the spring. However, there is a high variability in fruit production (Salvo et al. 1986; Dalmasso and Aconetani 1993). Some populations may bear a large amount of fruits in one season, whereas in others their production may be nil (Mooney et al. 1977), but the reason for these great differences in

Table 1. Review of the extent of seed predation by different species of bruchid beetles on *Prosopis* species of drylands of America.**Tabla 1.** Depredación de semillas por distintas especies de brúquidos, en especies de *Prosopis* de tierras secas de América.

<i>Prosopis</i> species	Bruchid species	Seed predation (%)	Region	Source
<i>P. velutina</i>	<i>Algarobius prosopis</i> , <i>Mimosestes amicus</i> , <i>M. protractus</i> , <i>Neltumius arizonensis</i>	<90	Sonora Desert, Mx	Kingsolver et al. 1977, Kistler 1995
<i>P. glandulosa</i>	<i>Algarobius prosopis</i> , <i>A. bottimeri</i>	90	Sonora Desert, USA	Zimmermann 1991
<i>P. laevigata</i>	<i>Algaronius johnsoni</i>	44	Chihuahua Desert, Mx	Salas Araiza et al. 2001
<i>Prosopis</i> spp.	<i>Algarobius prosopis</i>	48.5	Durango Desert, Mx	Solorio et al. 2004
<i>Prosopis</i> spp.	<i>Algarobius prosopis</i>	48.5	Durango Desert, Mx	Solorio et al. 2004
<i>P. caldenia</i>	Unidentified species	35	Espinal semidesert, La Pampa, Ar	Lerner and Peinetti 1996
<i>P. ferox</i>	<i>Scutobruchus feroxis</i>	25	Cardonal, Salta, Ar	Ortega Baes et al. 2001
<i>P. denudans</i>	<i>Rhipibruchus prosopis</i>	24	Monte Desert, Chubut, Ar	Cariaga et al. 2005
<i>P. alpataco</i>	<i>Rhipibruchus prosopis</i>	87	Monte Desert of Chubut, Ar	Cariaga et al. 2005
<i>P. chilensis</i>	<i>Scutobruchus ceratioborus</i> , <i>Scutobruchus</i> spp., <i>Rhipibruchus picturatus</i>	13.4-90*	Monte Desert, Catamarca, Ar	Solbrig and Cantino 1975
<i>P. exuosa</i>	<i>Scutobruchus ceratioborus</i> , <i>Scutobruchus</i> sp., <i>Rhipibruchus picturatus</i>	2.7-26.1**	Monte Desert, Catamarca, Ar	Solbrig and Cantino 1975
<i>P. chilensis</i>	<i>Scutobruchus</i> sp., <i>Rhipibruchus</i> sp.	90	Monte Desert, Catamarca, Ar	Kingsolver et al. 1977
<i>P. exuosa</i>	<i>Scutobruchus ceratioborus</i>	26	Monte Desert, Catamarca, Ar	Kingsolver et al. 1977
<i>P. chilensis</i>	<i>Scutobruchus</i> sp., <i>Rhipibruchus</i> sp., <i>Rhipibruchus</i> sp.	13	Monte Desert, Mendoza, Ar	This study
<i>P. exuosa</i>	<i>Scutobruchus ceratioborus</i> , <i>Rhipibruchus</i> sp.	5	Monte Desert, Mendoza, Ar	This study

The values correspond to two measurements, the first immediately after the fall of pods and the second, after the pods had spent 13 weeks on the ground beneath the parent plant.

** The values correspond to two measurements, the first immediately after the fall of pods and the second, after the pods had spent 15 weeks on the ground beneath the parent plant.

seed production remains unknown. In species of *Prosopis*, only 20-45% of initiated fruits reach full size (Cariaga et al. 2005). After that, in *P. flexuosa* and *P. chilensis*, it has been suggested that the fruits fallen on the ground are usually more preyed upon by insects (particularly bruchid beetles) than those remaining on the parent plant (Solbrig and Cantino 1975), however most of the studies on *Prosopis* have measured seed predation only in fruits still attached to tree crowns (e.g., Lerner and Peinetti 1996; Cariaga et al. 2005; Palleres 2007; Vega Riveros et al. 2009), which can represent an underestimation of seed predation.

The genus *Prosopis* includes shrubs and trees that fulfil important roles in both production and protection of ecosystems in many arid and semiarid regions of the world (Mares et al. 1977; Kingsolver et al. 1977; Golubov et al. 2001; Or and Ward 2003; Larrea-Alcázar et al. 2005; Hall and Hamilton 2014). In South America, open dry forest of *P. flexuosa* (D. C.) and *P. chilensis* (Molina) represent systems offering the opportunity to mitigate climate change and desertification, supporting the conservation of biodiversity and ecosystem services that underpin human livelihoods

(Villagra et al. 2009; Bastin et al. 2017). These trees are considered key species because they facilitate the occurrence of many other species under their crowns (Rossi and Villagra 2003; Rossi 2004; Larrea-Alcázar et al. 2005; Villagra and Álvarez 2006; Cesca et al. 2012; Greco et al. 2013). Moreover, *Prosopis* establishment increases soil fertility, nutrient cycling and dune stabilization (Aiazzi et al. 1996; Páez and Marco 2000; Álvarez et al. 2009; Miner et al. 2010; Cesca et al. 2012; Aranibar et al. 2014). Flowers represent resources for many insect species that pollinate them (Chacoff et al. 2018) and pods are an important food for native and exotic vertebrates (Mares et al. 1977; Campos and Ojeda 1997; Campos et al. 2007, 2008) which contributes to natural regeneration of forest by mutualistic seed dispersal (Campos and Ojeda 1997; Campos et al. 2007, 2008).

Considering the strong historical degradation that the open dry forests of *Prosopis* suffer (Villagra et al. 2009), estimation on the magnitude of pre-dispersal seed loss allows us to understand the role of this process as potential filter in seed dispersal and ultimately its implications to *Prosopis* population dynamics. The main goal of this study was to assess pre-

dispersal seed loss due to seed abortion and seed predation by insects in two *Prosopis* species from the Monte Desert: *P. flexuosa* and *P. chilensis*. The specific goals were 1) to identify, quantify and compare the sources of pre-dispersal seed loss in unripe and ripe pods; and 2) to explore the effects of the location of ripe pods in relation to the parent plant (crown and ground) on insect predation of seeds.

MATERIALS AND METHODS

Study species

Prosopis flexuosa is an arboreal legume that occurs in arid regions of Argentina and the central north of Chile, occupying vast sectors of the South American arid diagonal (Kingsolver et al. 1977; Álvarez and Villagra 2009). *Prosopis flexuosa* flowering occurs in October-November (mid-spring) and fruits ripen in December-February. Fruits are highly nutritive indehiscent pods, with a thin epicarp and a spongy mesocarp rich in carbohydrates (Karlin et al. 1997). Seeds (24-40 mg) have a high viability (98%) and germination capacity (100%) (Coni and Trione 1996; Campos et al. 2008). They are protected inside a woody endocarp and have an impermeable coat that causes physical dormancy (Catalán and Balzarini 1992). Once they are ripe, the fruits of *P. flexuosa* fall to the ground and accumulate under the parent plant. Even though this first movement may be interpreted as part of the seed dispersal syndrome, we considered it as a "presentation" of the fruit and not as primary dispersal (sensu Vander Wall et al. 2005). According to these authors, in species presenting a fruit structure typically related to zoochorous dispersal, primary dispersal is considered to be the first seed movement mediated by an animal.

Prosopis chilensis has been rarely studied from an ecological point of view. Native to South America, it is found from the south of Peru to nearly the parallel 34° S, being very abundant in the north and center of Chile. In Argentina, *P. chilensis* occurs in Monte, Espinal and Dry Chaco. Between 26° and 34° S, it coexists with *P. flexuosa*, which is much more abundant (FAO 2000). *Prosopis chilensis* is one of the species with the highest phenotypic variation (Burkart 1976; Contreras Arellano 1984; Karlin et al. 1997). Indehiscent pods ripen at same time as those of *P. flexuosa* (Silva et al. 2000), but are less nutritious for lacking the spongy and sweet mesocarp, and

the woody endocarp (instead, they have a thin leathery endocarp). Seeds have a high viability (90%) and germination capacity (80%) (Coni and Trione 1996; Campos et al. 2008).

Study area

The Monte Desert is the most arid ecoregion of Argentina (Fernández and Busso 1997) that, covers 38 million hectares and crosses the country diagonally (Roig-Juñet et al. 2001). The climate is semiarid to arid with high evaporation rates. Annual rainfalls (80 to 450 mm) occur primarily in the summer and mean annual temperature varies between <10 and 18 °C (Abraham et al. 2009). Some remnants of *Prosopis* woodlands are included in the two protected areas where this study was conducted: The Man and Biosphere Ñacuñán Reserve in the Central Monte and the Ischigualasto Provincial Park in the Northern Monte. The Ñacuñán Reserve (34°02' S - 67°58' W) is located in Mendoza Province and it is the only fenced protected area in the Monte free from livestock since 1972. The reserve encompasses 12800 ha and it has an average annual precipitation of 326 mm. After a 50-year grazing exclusion, the passive recovery of the native vascular flora is remarkable. In this area, the major plant communities are open woodlands of *P. flexuosa* within a matrix of xerophytic shrubs (Roig and Rossi 2001). The Ischigualasto Park (29°55' S - 68°05' W), where we studied *P. chilensis*, is located in San Juan Province, within a region where average annual precipitation in the area is 183 mm. The reserve encompasses 62916 ha and has a very low density of domestic herbivores (C. Campos, unpublished data).

Field sampling

To assess pre-dispersal seed loss in *P. flexuosa*, we worked on two plots of variable sizes (1-3 ha) separated by 2 km. This variation in plot size responded to the requirement of obtaining independent samples from randomly selected adult trees (with criteria DBH 20-40 cm) that produce fruits (not all individuals in a plot produce fruits). The minimum pairwise distance between the trees was 15 m because bruchid species have a low flight capacity and go through their full life cycle generally on the same tree (S. Muruaga de L'Argentier, personal communication). Individuals trees were sampled in two plots and study was carried out in two consecutive fruiting seasons (2007-2008).

In order to identify and quantify pre-dispersal seed loss by insect predation in unripe pods of *P. flexuosa*, we collected 5 pods from different points of the crown of 16 individuals (8 per plot) in December 2007. Unripe pods are defined by being elongated, uniformly green in color, with 2-mm preformed seeds (Cariaga et al. 2005). The number of pods collected was limited because fruit ripening is not fully synchronized among individuals, and five was the sample size that fulfilled the "immature" condition at the time of sampling. In this summer, the migratory burrowing parrot (*Cyanoliseus patagonus*) and monk parakeet (*Myiopsitta monacha*) arrived at the same time of the beginning of fruit ripening, consuming seeds from unripe pods on the trees (S. Velez, personal observation); so, this also limited the number of unripe pods per sample.

To identify and quantify pre-dispersal seed loss by abortion and insects' predation in ripe pods of *P. flexuosa*, we collected 10 pods per tree, from different points of the crowns up to 2 m in height of 40 individuals located into the two plots (17 individuals in 2007 [10 from plot 1 and 7 from plot 2] and 23 in 2008 [10 from plot 1 and 13 from plot 2]). To explore the effects of pod location on seed predation by insects, we collected 10 pods from the ground at the same time of crown samples. We obtained 33 ground samples instead 40 (12 individuals in 2007 [6 from plot 1 and 6 from plot 2] and 21 in 2008 [8 from plot 1 and 11 from plot 2]) because it was not always possible to find healthy pods, not disaggregated or not partially preyed upon by

parrots, sigmodontine rodents or ants (Milesi and Lopez de Casenave 2004).

To identify and quantify pre-dispersal seed loss by abortion and insects' predation in ripe pods of *P. chilensis*, we worked on two 2-ha plots located 5 km apart (due to natural distribution of *P. chilensis* forest in Ischigualasto). We collected 10 pods from different points of the crown of 31 individuals in the two plots (19 in 2007 [9 from plot 1 and 10 from plot 2] and 12 in 2008 [from plot 1]). To explore the effects of pod location on seed predation by insects, sampling was carried out in the same way as for *P. flexuosa*. In 2008, it was not possible to find pods on the ground due to large storms which dragged everything from the ground surface; thus, we obtained 31 crown samples and 21 ground samples.

Laboratory procedures

To assess the source of seed loss (seed abortion or insect predation), all samples collected were placed in individual transparent PVC containers (500 g) and stored under constant temperature (20 °C) and natural photoperiod. For the analysis of data, we considered the total seed number per 10-pods sample, for *P. flexuosa* was 173 (± 23 SD) and 236 (± 39 SD) for *P. chilensis*.

Pods were left for an incubation period of 50 days to allow development of all the insects that were potentially infecting seeds at sampling time, as the entire life cycle (from egg to adult) of the bruchid species cited for



Figure 1. *Prosopis flexuosa* seeds predated by bruchid beetles in different stages of its life cycle. a) Predated seed by born adult and endocarp with characteristic adult exit hole. b) Seed without endocarp with an unborn adult bruchid beetle inside. c) Seed without endocarp with a pupa of bruchid beetle; d) Seed without endocarp with an advanced larva of bruchid beetle.

Figura 1. Semillas de *Prosopis flexuosa* depredadas por brúquidos en distintos estadios de su ciclo de vida. a) Semilla depredada por un brúquido que emergió por el orificio característico dejado sobre el endocarpio por el adulto. b) Semilla sin endocarpio depredada por un brúquido adulto no emergido aún en su interior. c) Semilla sin endocarpio con una pupa de brúquido. d) Semilla sin endocarpio con una larva de brúquido en los últimos estadios.



Figure 2. *Prosopis flexuosa* seeds predated by apionid weevils. Adult exit holes on pod epicarp (left), and on endocarp (middle and right).

Figura 2. Semillas de *Prosopis flexuosa* depredadas por apiónidos. Orificio de emergencia del adulto sobre el epicarpio (izquierda) y sobre el endocarpio (medio y derecha).

P. flexuosa and *P. chilensis* in other areas of the Monte Desert is 28-50 days (Muruaga de L'Argentier 1986). All samples were checked every 3 days in order to record emerging insects and remove them to prevent mating and reinfection inside the containers. All pods were processed after the incubation period. Unripe and ripe pods were first externally inspected to detect and quantify signs of insect seed damage by comparing number of exit holes with insect births during the incubation period. Then, the pods were opened with metallic pliers to extract all seeds, which were individually examined and classified into different categories according to their status. For unripe fruits, we found two sources of loss: seeds preyed upon by apionid weevils and seeds preyed by bruchid beetles.

For ripe fruits, we found five different sources of seed loss, with subcategories: 1) seeds preyed upon by bruchid beetles (seeds with a characteristic 1.6-2 mm oval exit hole left by the adult [Figure 1a]), seeds with an unborn adult (Figure 1b), seeds with pupae (Figure 1c), or seeds with an advanced larva (Figure 1d); 2) seeds preyed upon by apionid weevils (seeds with a characteristic 0.5-1.1 mm oval exit hole) (Figure 2). These seeds can also be recognized before removing the epicarp and endocarp because they are significantly thinner than those preyed upon by bruchid beetles. Inside the endocarp there is almost no or very scarce seed material; 3) seeds preyed on by unidentified insects (flattened and dark seeds that get broken into small pieces) (Figure 3a). In some cases, there was no seed inside the endocarp; instead, there were remnants of dark powder, very small cotyledon pieces (Figure 3a, top part), or remnants of cocoon-like material (Figure 3a, bottom part). Pods with this type of seed predation often have tiny punctures on the epicarp (Figure 3b); 4) aborted seeds (the external appearance of the endocarp is flattened like the seeds inside it, which generally are dark brown in color (Figure 3c, top part), or sometimes light yellow); 5) intact seeds (seeds with no apparent signs of seed predation [no exit holes, or depressions, marks, spots or powder on seed coats], regular color and shape [Figure 3c, bottom part]). Although taxonomic identification of seed predators was not among our goals, we explored the identity of insect species born in PVC containers and bruchid species were identified (see Acknowledgements).



Figure 3. *Prosopis* pods and seeds predated by unidentified insects. a) *P. flexuosa* endocarp with piece of seeds (top part) and with rest of cocoon material (bottom part). b) *P. chilensis* pod with tiny punctures on epicarp. c) *P. flexuosa* aborted (top part) and intact seeds (bottom part).

Figura 3. Frutos y semillas de *Prosopis* depredadas por insectos no identificados. a) Endocarpio de una semilla de *P. flexuosa* con pequeños restos de endosperma y cubierta seminal (parte superior de la imagen) y con restos de material parecido a un capullo (parte inferior de la imagen). b) Frutos de *P. chilensis* con pequeñas punciones en el epicarpio. c) Semillas de *P. flexuosa* abortadas (parte superior de la imagen) y semilla sana de dimensiones y coloración normal (parte inferior de la imagen).

Statistical analysis

All analyses and graphs were built with R language and environment (R Core Team 2014). To compare the relative importance of sources of seed loss in each *Prosopis* species, we made the analysis using five response variables: mean proportion per tree of seeds preyed upon by bruchid beetles (the variable correspond to the proportion of seeds that are preyed on 10 pods), by apionid weevils, by unidentified insects, total seed loss by insect predation (which is the sum of the first three variables), aborted seeds, and total seed loss (sum of seed loss by insect predation and aborted seeds). Since all these response variables were taken on the same sample (10 pods per tree), we could not consider them independent; therefore, we used nonparametric procedures with different functions provided by the nparcomp package (Konietschke 2012; Konietschke et al. 2014). With this package it is possible to compute nonparametric simultaneous confidence intervals for relative contrast effects and p-values in the unbalanced one-way design. There is no assumption on the underlying distribution function, only that the data have to be at least ordinal.

We compared the two sources of seed loss in unripe fruits found in *P. flexuosa* (seed predation by apionid weevils vs. seeds predation by bruchid beetles) by using a nonparametric paired t-test procedures provided by the npar.t.test.paired function (package nparcomp). The function performs a two sample studentized permutation test for paired data, that is testing the hypothesis $H_0: p=1/2$, where p denotes the relative effect of 2 dependent samples, and computes a confidence interval for the relative effect p. In addition, the Brunner-Munzel-Test was accompanied by a confidence interval (Munzel and Brunner 2002; Konietschke and Pauly 2012).

We compared among different sources of seed loss in ripe fruits by using the mctp.rm

function (package nparcomp) (Konietschke et al. 2010). This function computes the estimator of nonparametric relative effects based on global rankings, simultaneous confidence intervals for the effects and adjusted P-values based on Tukey’s contrasts in a repeated measure design (dependent variables). For this analysis we used a numeric response variable (mean proportion for total seed loss per tree) and a repeated measures factor with four levels: seeds preyed on by bruchid beetles, by apionid weevils, by unidentified insects, and aborted seeds. To compare total seed loss by insect vs. seed loss by abortion in ripe pods we used a nonparametric paired t test procedure provided by the npar.t.test.paired function, as we have described previously.

To assess the effect of pod location (crown or ground) on seed predation by insects on ripe pods of both *Prosopis* species, we compare seed loss in the crown with seed loss in the ground by each group of insects. We performed the npar.t.test with the proportion of seeds per tree preyed upon by each group of insects (bruchid beetles, apionid weevils and unidentified insects) as response variable, and pod location as grouping factor with two levels (crown and ground).

We checked the variability of samples between plots and years of sampling with ANOVA, using as response variable the proportion of seed loss by different sources (grouping seed loss in crown and on the ground), and plots and years as fixed factors with two levels. Considering there were no differences in total seed loss between years (*P. flexuosa*: $F=3.139$, $P=0.10$; *P. chilensis*: $F=1.543$, $P=0.22$) or plots (*P. flexuosa*: $F=1.420$, $P=0.24$; *P. chilensis*: $F=0.155$, $P=0.70$) each tree was considered a replicate and we used percentages to describe general results.

RESULTS

We analyzed a total of 12905 seeds of *P. flexuosa*, 280 seeds in unripe pods and 12625 seeds in ripe pods. Pre-dispersal seed loss

Table 2. Sources of seed loss (mean percentage ± standard error) in different ripeness stages of two *Prosopis* species. Each variable was obtained from the average of crown and ground samples per tree between.

Tabla 2. Fuentes de pérdida de semillas (porcentaje medio ± error estándar) en distintos estados de maduración del fruto de dos especies de *Prosopis*. Cada variable fue obtenida a partir del promedio entre copa y suelo para cada árbol.

Source of seed loss	<i>Prosopis flexuosa</i>		<i>Prosopis chilensis</i>
	Unripe stage	Ripe stage	Ripe stage
Seed loss by apionid weevils	10.33 (±21.51)	9.62 (±8.64)	0
Seed loss by adults bruchid beetles	1 (±3.95)	3.67 (±3.33)	6.48 (±3.97)
Seed loss by unborn pre-imaginal stages of bruchid beetles	0	1.4 (±1.32)	6.23 (±4.82)
Seed loss by unidentified insects	0	4.92 (±3.70)	2.85 (±2.19)
Aborted seeds	0	19.94 (±7.24)	7.22 (±4.11)

Table 3. Multiple non-parametric comparison among different sources of seed loss. Tukey's pseudo-rank and contrast estimation method, 95% confidence level.**Tabla 3.** Comparación múltiple no paramétrica entre las diferentes fuentes de pérdida de semillas identificadas. Método de estimación por pseudo rangos y contraste de Tukey, nivel de confianza del 95%.

	<i>Prosopis flexuosa</i>						<i>Prosopis chilensis</i>		
	Unripe stage			Ripe stage			Ripe stage		
	Estimator	Statistic	P-value	Estimator	Statistic	P-value	Estimator	Statistic	P-value
Aborted seeds- Apionid weevils	-	-	-	-0.202	-3.170	0.008	-	-	-
Aborted seeds- Bruchid beetles	-	-	-	-0.367	-8.468	<0.001	0.208	4.293	<0.001
Aborted seeds- Unidentified insects	-	-	-	-0.333	-7.525	<0.001	-0.341	-10.05	<0.001
Apionid weevils- Bruchid beetles	0.316	-3.006	0.016	-0.165	-2.531	0.05	-	-	-
Bruchid beetles- Unidentified insects	-	-	-	0.035	0.794	NS	-0.549	-18.55	<0.001

caused by insect predation in unripe pods was 12% of produced seeds, caused by seed predation by insects. We could not recognize aborted seeds at this ripeness stage. We identified two sources of seed loss in unripe *P. flexuosa* pods: seed predation by apionid weevils and seed predation by bruchid beetles (Table 2). We could not recognize aborted seed at this ripeness stage. Seed predation by apionid weevils was significantly more important than seed predation by bruchid beetles, which was very low (Table 2 and Table 3).

Pre-dispersal seed loss in ripe pods of *P. flexuosa* was 32% of produced seeds. Seed loss caused by insects was 19% of produced seeds. We identified four sources of seed loss in ripe pods of *P. flexuosa* (Table 2): seed predation by apionid weevils, seed predation by bruchid beetles, seed predation by unidentified insects, and seed abortion. The proportion of aborted seeds was significantly higher than any of the proportions of seed loss (Table 3), except when total seed loss by insects is considered ($T=3.928$, $P=0.001$). The proportion of seeds preyed upon by apionid weevils was higher than seeds preyed upon by bruchid beetles (marginally significantly) or by unidentified insects (Table 3). An important result related to bruchids was the presence of unborn pre-imaginal stages inside predated seeds (Table 2 and Figure 1b, c, d), even when they had time required to complete their life cycle. Predation by unborn pre-imaginal stages of bruchid beetles represented 41% of total seed predation by bruchid beetles.

We analyzed 12249 seeds of ripe pods of *P. chilensis*, and pre-dispersal seed loss was 21%

of produced seeds. Seed predation by insect was 14% of produced seed. We identified three sources of seed loss in ripe pods of *P. chilensis* (Table 2): seed predation by bruchid beetles, seed predation by unidentified insects, and seed abortion. No seed predation by apionid weevils was found in this species. The proportion of seeds preyed upon by bruchid beetles was significantly higher than the proportion of seed loss by other sources (Table 3). As in *P. flexuosa*, a high proportion of *P. chilensis* seeds was infected by unborn pre-imaginal stages of bruchid beetles (Table 2 and Figure 1b, c, d) representing 49% of total seed predation by bruchid beetles.

Pod location had no significant effect on seed predation by insects on either *Prosopis* species. Seed predation by bruchid beetles was not different between crown and ground (*P. flexuosa*: $T=-1.115$, $P=0.26$; *P. chilensis*: $T=-1.009$, $P=0.16$), neither was it different for apionid weevils (*P. flexuosa*: $T=1.42$, $P=0.16$), or for unidentified insects (*P. flexuosa*: $T=-0.421$, $P=0.67$; *P. chilensis*: $T=-0.492$, $P=0.62$).

Of all bruchid specimens examined, 95% belonged to the genus *Scutobrachus*. In *P. flexuosa*, the most abundant species was *S. ceratioborus*. Another genus present in a very small proportion was *Rhipibruchus*. In *P. chilensis*, the genus *Scutobrachus* was the most conspicuous and *Pectinibruchus* and *Rhipibruchus* were present in a lower proportion. Another group of insects found in pod containers and inside seeds of ripe pods were unidentified species of microhymenoptera, which probably were parasites of seed beetle larvae.

DISCUSSION

The reproductive potential of plants diminishes progressively from ovule to seedling because of multiple biotic and abiotic factors acting in conjunction or successively. During the pre-dispersal phase of two *Prosopis* species, we examined 25154 seeds from different trees during two consecutive fruiting seasons and found that pre-dispersal seed loss due abortion and insect predation is about 20-30% of all seeds produced. In both *Prosopis* species studied, the main cause of pre-dispersal seed loss was predation by insects. The most important taxa in terms of magnitude were apionid weevils for *P. flexuosa* and bruchid beetles for *P. chilensis*.

Seed predation by bruchid beetles on the *Prosopis* species studied in this work is relatively low compared to other *Prosopis* species inhabiting drylands of the northern and southern hemispheres (Table 1). This study showed that predation by bruchid beetles on *P. flexuosa* was similar to that found at the end of the fruiting season by another study of the same species but inhabiting the northern portion of the Monte Desert (Solbrig and Cantino 1975). Our study found that predation by bruchid beetles on *P. chilensis* was relatively low compared to that found by Solbrig and Cantino (1975) for *P. chilensis* at the end of the fruiting season. In addition to the differences between the geographical areas of the study sites and probably the insect guild seed predators, the comparisons may not be entirely accurate since each study used different methodologies for making estimates. For instance, estimations of seed predation were done immediately after the fall of fruits in some cases and after the fruits had spent several weeks on the ground beneath the parent plant in other cases. Even so, several authors (e.g., Janzen 1971; Anderson 1988) claim that estimates of insect pre-dispersal seed predation are still conservative or even misleading due to inadequacy of the sampling methodology.

Rates of pre-dispersal seed predation are usually estimated by inspecting ripe fruits and seeds for signs of insect damage; however, this method may seriously underestimate the real losses (Anderson 1988). The methods and procedures used by our work revealed that total predation by bruchid beetles includes two proportions, a proportion of seeds preyed on by bruchid beetles that had completed their life cycle (exit hole count method), and another proportion preyed on by individuals

still inside the seed. This indicates that even having had enough time to develop the two typical generations that commonly occur in nature, permanence of insects inside the seed is possible. This could be because insects die during development (Muruaga de L'Argentier 1986) or naturally enter into diapause at the end of summer. This proportion of unemerged insects represented 41% of seeds damaged by bruchids in *P. flexuosa* and 49% in *P. chilensis*, showing the importance of method selection for a realistic estimate of seed predation by beetles.

This work highlights the role of apionid weevils as pre-dispersal seed predators of unripe fruits of *P. flexuosa*. Until now, only *Coelocephalapion gandolfoi* K. (Brentidae: Apioninae) has been identified as a pre-dispersal seed predator of *Prosopis* (Kissinger 2005; Mc Kay and Gandolfo 2007). Probably, this is a univoltine species with one generation by fruiting season (Mc Kay and Gandolfo 2007), a characteristic behavior of species infecting unripe green fruits and seeds (Schmidt 1998). According to Mc Kay et al. (2012), quantifying seed predation by *C. gandolfoi* on unripe pods could have been overestimated since unripe infected pods contained early stages of the weevil (eggs or newly emerged larvae) which may not develop later. Assessing seed predation on ripening pods represented a more realistic estimate because pods and seeds were almost fully developed and no longer suitable for oviposition. Our results showed that our methodology allowed development of early weevil stages in unripe pods, obtaining similar seed predation estimates for unripe and ripe pods.

In both *Prosopis* species, we observed about 3-5% of seeds preyed upon by unidentified insects. This group could be composed of different insects that have been observed crossing immature and mature fruits, branches, leaves and ground beneath *Prosopis* crowns, like ants, beetles, parasites (Hymenoptera, Diptera) and sucking insects (Hemiptera, Heteroptera), suggesting that the arthropod community that interacts with *Prosopis* fruits may be broader than is currently known (Flores et al. 2004; Mc Kay and Gandolfo 2007).

Seed loss due to abortion was greater than insect seed predation in the case of *P. flexuosa*. Seed abortion is, in many plants, the main cause of loss of reproductive structures in the pre-dispersal phase, which greatly limits the amount of seeds produced. Seed abortion

have different ecological and evolutionary origins (Stephenson 1981), and it can occur as a physiological response to severe environmental stress (Janzen 1971, 1977; Udovic and Aker 1981). In this study the mechanisms underlying the loss of seeds by abortion were not explored but, considering the importance of seed loss by abortion, it needs to be carefully studied in future works.

In both tree species studied, the location of fruits in relation to the parent plant did not affect seed predation by bruchid beetles, or by the other insect groups. This may indicate that bruchid beetles have no preference for the location of fruits for their oviposition. Under natural conditions, once all the fruits have fallen to the ground, these non-visible bruchid beetles that are actually infecting seeds could continue pod seed infection and predation on the ground if the pods are not removed by vertebrates (Ortega Baes et al. 2001). In the long term, this could result in the destruction of many produced seeds for bruchid generations that may follow, as weather conditions permit (Solbrig and Cantino 1975; Ortega Baes et al. 2001).

In this study, we quantified seed loss of pre-dispersal stages due to abortion and insect predation. However, other causes of pre-dispersal seed predation were not estimated. For instance, seed consumption by birds can be an important source of pre-dispersal seed loss (Milesi and Lopez de Casenave 2004). During

summer of 2007-2008, we observed abundant flocks of burrowing parrot (*Cyanoliseus patagonus*) and monk parakeet (*Myiopsitta monacha*) in the Ñacuñán Reserve, but we have not observed their presence in our sample plots during others years of work. Although the presence and abundance of these species in the study area can be very spatially and temporally variable (Bucher and Rinaldi 1986; Marone 1992; Pruett-Jones and Tarvin 1998; Masello et al. 2006; Masello et al. 2011; Masello and Quillfeldt 2012; Barría et al. 2017), their feeding activity on unripe pods is an unknown side of pre-dispersal seed loss that could be important for *Prosopis* species.

Prosopis species are key species in drylands, because of their relationships with both plant and animal communities, and for the livelihoods of many ancient and modern human groups (Kingsolver et al. 1977; Mares et al. 1977; Felker 1981; Or and Ward 2003; García-Sánchez et al. 2012). After the degradation of millions of hectares of *Prosopis* forests in Argentina (Verga 2000; Rojas et al. 2009), understanding seed loss process may contribute to know and predict plant population dynamics (Kolb et al. 2007), revealing the natural regeneration mechanisms to forest recovery.

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