REVISTA CHILENA DE HISTORIA NATURAL

Revista Chilena de Historia Natural 84: 289-299, 2011

RESEARCH ARTICLE

Relationships between *Prosopis flexuosa* (Fabaceae) and cattle in the Monte desert: Seeds, seedlings and saplings on cattle-use site classes

Interacciones entre *Prosopis flexuosa* (Fabaceae) y el ganado en el desierto de Monte: Semillas, plántulas y renovales en los sitios de uso del ganado

CLAUDIA M. CAMPOS[1, 2, 3, *], VALERIA E. CAMPOS[2, 3], ARNALDO MONGEAUD[4], CARLOS E. BORGHI[2, 3], CLAUDIA DE LOS RÍOS[3] & STELLA M. GIANNONI[2, 3]

[1] Grupo de Investigaciones de la Biodiversidad, Instituto Argentino de Investigaciones de las Zonas Áridas, CC 507, 5500, Mendoza, Argentina

[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

[3] Instituto y Museo de Ciencias Naturales, Universidad Nacional de San Juan, Departamento de Biología (INTERBIODES)

[4] Universidad Nacional de Córdoba, Argentina

*Corresponding author: ccampos@lab.cricyt.edu.ar

ABSTRACT

The fate of *Prosopis flexuosa* seeds dispersed by cattle is dependant on the spatial pattern of dung deposition and foraging movements. We hypothesised that cattle-use site classes explain the response variables related to seed input and fate of seeds, seedlings and saplings (small plants more than one year old). We defined sites with heavy cattle traffic (“trails” and “periphery of trails”), sites used for resting and foraging (“under *Prosopis*”), and sites where isolated individuals only walk (“under shrubs” and “bare soil”). Considering the established cattle-use site classes, our specific goals were to quantify and compare: (1) seeds transported in cattle dung; (2) seedlings 10 months after dung deposition; (3) established saplings; and (4) germinated and remaining seeds, and seedling survival in dung immediately after dung deposition. In a grazed field at Ñacuñán (Mendoza, Argentina) we worked in four similar areas, each consisting of 25-ha plots 2 km apart. Space use by cattle caused differential seed input: “under *Prosopis*” and in the “periphery of trails” animals deposited the largest amounts of dung and seeds. Ten months after dung deposition, the highest number of seedlings occurred on “trails”, “under *Prosopis*” and in the “periphery of trails”. In the long term, the highest number of established saplings occurred only in the “periphery of trails”. The number of seeds germinated immediately after fruit production and dung deposition was very low. Survival of seedlings sprouting from dung-germinated seeds did not exceed one week. On “trails” and in the “periphery of trails” the persistence of seeds in dung was low because of dung disintegration by the action of cattle trampling. The seeds that did not remain in dung were probably the source of seeds that will germinate in the next wet season (i.e. 10 months after dung deposition). With different effects depending on cattle site activity and on the stage of the *P. flexuosa* plant (seed, seedling, or sapling), defecation and trampling appear to be important processes in the seed dispersal cycle. In this sense, cattle could benefit the establishment of *P. flexuosa*.

Key words: dung deposition, endozoochory, establishment, germination, seed input.

RESUMEN

El destino de las semillas de *Prosopis flexuosa* dispersadas por ganado depende de los patrones de deposición de excrementos y movimientos de forrajeo. Nuestra hipótesis es que las variables respuesta relacionadas con el aporte y el destino de semillas, plántulas y renovales son explicadas por diferencias entre los sitios de actividad del ganado. Definimos sitios con alto tránsito de animales (“senderos” y “periferia de senderos”), sitios utilizados para descanso y alimentación (“bajo *Prosopis*”) y sitios de paso para animales aislados (“bajo arbustos” y “suelo descubierto”). Considerando los sitios de actividad establecidos, nuestros objetivos fueron cuantificar y comparar: (1) semillas transportadas en los excrementos, (2) plántulas 10 meses después de la deposición, (3) renovales establecidos, (4) semillas germinadas en los excrementos, supervivencia de plántulas y semillas que permanecieron en los excrementos. El trabajo se realizó en un campo ganadero de Ñacuñán (Mendoza, Argentina), dentro de cuatro áreas donde se establecieron parcelas de 25 ha. En los sitios se encontró un aporte diferencial de semillas: en sitios de descanso y alimentación (“bajo *Prosopis*”) y de tránsito intenso (“periferia¨) los animales depositaron las mayores cantidades de semillas. Diez meses después, las semillas germinaron con las primeras lluvias; el mayor número de plántulas se registró en sitios de tránsito intenso (“senderos” y “periferia”) y de descanso y alimentación (“bajo *Prosopis*”). A largo plazo, en la “periferia” se establece la mayor cantidad de renovales. Inmediatamente después de la deposición, germinó un número muy bajo de semillas y las plántulas no sobrevivieron más de una semana. En los sitios más transitados por el ganado, los excrementos se desintegraron por pisoteo y la persistencia de las semillas en los excrementos fue baja o nula. Probablemente, las semillas que retornaron al suelo luego de la desintegración de los excrementos son las que germinaron 10 meses después. Con diferentes efectos, dependiendo del sitio de actividad del ganado y el estado de vida de la planta (semilla, plántula, renoval), la defecación y el pisoteo parecen ser procesos importantes en el ciclo de dispersión. En este sentido, el ganado podría beneficiar el establecimiento de *P. flexuosa*.

Palabras clave: aporte de semillas, deposición de excrementos, endozoocoria, establecimiento, germinación.

INTRODUCTION

Seed dispersal is the emigration of seeds away from the parent plant. However, it is not the movement itself that is beneficial for the plant and its seeds, but rather the movement toward particular sites where seeds have at least some chance of growing into an adult plant (Janzen 1983). For this reason, over the last two decades, numerous studies have evaluated how mammals may affect seed dispersal and seedling establishment through patterns of seed deposition, foraging movement and behaviour (Stoner et al. 2007).

Dung deposition and trampling affect local colonization processes by enhanced propagule dispersal (dung as a source of colonizers in the form of seeds) and by creation of gaps with potential favourable conditions for germination and seedling growth (high light, nutrient-rich and pathogen-free soil; Olff & Ritchie 1998). It is during pre-germination and seedling establishment (germination and first year of life; Clark et al. 1999) that most mortality occurs (Janzen 1970, Harper 1977, Howe & Smallwood 1982, Kitajima & Fenner 2000, Wang & Smith 2002).

The usual fate of seeds excreted in dung is to germinate immediately, remain dormant in dung, be mixed into the litter by the action of dung decomposition (Janzen 1982, Malo & Suárez 1995a, 1995b, Dai 2000), or be removed by vertebrates and invertebrates (Janzen 1982). The dung that accompanies the seeds has potential to greatly influence the fate of dispersed seeds (Janzen 1986). Characteristics of faecal material such as toxicity for seedlings and low affinity for water can result in selective development of plants with ability to survive in faeces (Grace & Tilman 1990, Lobo & Veiga 1990). Also, faecal material attracts dung beetles, ants, and small rodents, which can act as predators or secondary dispersers of seeds contained in dung (see review by Andresen & Feer 2005, Vander Wall & Longland 2005).

If endozoochorously dispersed dung-borne seeds are not removed, seed germination and establishment will depend on safe-site availability (sensu Harper 1977). A safe site offers the appropriate biotic and abiotic conditions needed for seed germination and seedling establishment. Therefore, the potential effect of endozoochory on plants will depend on spatial patterns of defecation and on the number of seeds in the dung (Malo et al. 2000).

In arid zones of Argentina, *Prosopis* seeds are dispersed by native and non-native mammals through endozoochory. *Prosopis* seeds are included in pods and exhibit "animal dispersal syndromes" (Mooney et al. 1977). Seeds enter the digestive system of animals and can survive the passage through the animal's gut because they are wrapped in a hard and stony endocarp and an impermeable coat (Kingsolver et al. 1977). These structures maintain physical dormancy and protect seeds from the chemical and abrasive action in the digestive tract (Fenner & Thompson 2005). After animal digestion, seeds can lose viability and be subject to different effects on their germinability, depending on the particular mammal and *Prosopis* species involved (e.g., Eilberg 1973, Peinetti et al. 1992, 1993, Campos & Ojeda 1997, Ortega-Baes et al. 2002, Campos et al. 2008).

P. flexuosa seeds in the Monte desert, are dispersed by endozoochorous native mammals (such as *Pseudalopex griseus, Dolichotis patagonum, Lagostomus maximus, Lama guanicoe*), and non-native mammals (wild species: *Lepus europaeus* and *Sus scrofa*; domestic animals: cow, horse, donkey; Campos & Ojeda 1997, Campos et al. 2008). Overall, these dispersers decrease seed viability by up to 50 %, except for *Sus scrofa*, which kills nearly all seeds. In comparative studies of percent germination of seeds either collected directly from trees without scarification or ingested by animals, it was found that only two species enhanced germination significantly: *L. europaeus* and *L. guanicoe*. Cattle facilitated germination of only 10-15 % of seeds (Campos & Ojeda 1997, Campos et al. 2008).

The effect of cattle consumption on *Prosopis* seeds is herein studied because cattle are considered an important agent of *Prosopis* seed dispersal and *Prosopis* expansion (Fisher 1977, Brown & Archer 1987, 1989, Kramp et al. 1998). In North American grasslands, cattle have a key role facilitating *P. glandulosa* seed germination and plant establishment (Brown & Archer 1987, 1989, Kramp et al. 1998). Comparative studies of seed dispersal and establishment between areas without cattle and areas with a different grazing history showed that seeds are found away from trees only in the area with presence of cattle (Brown & Archer 1987, 1989). In arid zones of Argentina, cattle consume fruits of *P. caldenia* (Peinetti et al. 1992, 1993), *P. ruscifolia* (Eilberg 1973) and *P. flexuosa* (Campos & Ojeda 1997). Seed viability after cattle consumption is affected by up to 50 % (Eilberg 1973, Peinetti et al. 1993, Campos & Ojeda 1997), and seeds are primarily eliminated with no endocarps. The studies found low seed germination during the first month after seed consumption by cattle (Eilberg 1973, Campos & Ojeda 1997) even in seeds contained in dung (Peinetti et al. 1992).

Although over 50 % of *Prosopis* seeds usually survive after passage through the cattle gut, seed germination is low, and therefore endozoochory would not have a direct beneficial effect on seed germination. However, gut passage could provide other benefits, such as opening impermeable endocarps and freeing seeds, with more chances of physical scarification (Peinetti et al. 1992, Campos & Ojeda 1997, Kneuper et al. 2003). Also, seed movement by animals has a primary favourable effect on seeds (Janzen 1970) and then the fate of viable seeds will depend on the spatial pattern of cattle defecation (type and intensity of use of different areas by cattle, effect of dung deposition, and effect of trampling; Malo et al. 2000).

In this context, our hypothesis is that the benefits of *P. flexuosa* seed dispersal by cattle are associated with the site where dung with seeds is deposited. The activity and movement of cattle produce differences in seed input, fate of seed, seedling production and sapling establishment among cattle-use site classes.

We predict that sites with heavy cattle traffic (cattle-use site classes named “trails” and “periphery of trails”) and sites used for rest and forage (cattle-use site class named “under *Prosopis*”) will have higher input of seeds than sites where isolated individuals only walk (cattle-use site classes named “under shrubs” and “bare soil”). Also, after some time, at sites with heavy traffic of cattle and sites used for resting and foraging, because of disturbances produced by cattle trampling, the seeds would have more probabilities of scarification and then we expect that the number of seedlings will be higher. Finally, we predict that sapling establishment will also be high at sites intensively used by cattle to move, rest and forage. Related to these predictions, using an observational approach, our specific goals were: (1) to quantify and compare number of *P. flexuosa* seeds transported in cattle dung (input of seeds) to cattle-use site classes; (2) to compare number of seedlings among different sites 10 months after fruit production and dung deposition; (3) to compare number of established saplings among sites. Germination of dung-borne seeds immediately after dung deposition depends mostly on the potential scarification in the cattle digestive tract and on the humidity contained in dung. At this time, the site where dung was deposited is probably of little importance. Considering that the passage through the cattle gut itself does not significantly improve seed germination, we expect that seed germination immediately after dung deposition will be low at all sites. And, as in previous studies, we also predict a high mortality of seedlings in dung. Then, if few seeds germinate in dung, we can expect most of the seeds to disappear due to trampling or consumption/dispersal by animals (such as rodents, ants, beetles, birds). Using an experimental approach, our fourth specific goal was to compare, among cattle-use site classes, the number of germinated and remaining seeds and seedling survival in dung immediately after dung deposition.

METHODS

Study area

The study was conducted between February and April 2006, immediately after fruit production and during pod consumption by cattle and dung deposition, and in December 2006, once the dry season had ended and after the first rainfall events.

The study area is a grazed field located in Ñacuñán (Santa Rosa Department, Mendoza Province, Argentina), in the central part of the temperate Monte Desert (Morello 1958). Geomorphologically it is an undulating plain (540 masl), composed of fine-grained soils derived from the Andes Cordillera that lies 100 km to the west (Tanquilevich 1971). The climate is semiarid and markedly seasonal. Mean monthly temperatures range from < 10°C in winter to > 20 °C in summer (Ojeda et al. 1998). Average annual rainfall is 329.4 mm (Estrella et al. 2001). Total rainfall during the study period (February to December 2006) was 359.5 mm (mean monthly precipitation = 29.96 mm; SE = 9.10; range = 0-80). The heaviest rains occurred in February and December, while no rain fell in April, May, August or October. The major plant communities in this area are open woodlands of *P. flexuosa* that compose the tree layer along with specimens of chañar (*Geoffroea decorticans*), a shrub layer of jarilla (*Larrea divaricata* and *L. cuneifolia*) and piquillín (*Condalia microphylla*), and a grass and herb layer (Roig 1971).

At the beginning of the 19th century, the area of Ñacuñán suffered severe exploitation from indiscriminate logging of *Prosopis* woodlands, and its growing impoverishment led to intensified cattle breeding. Currently, the area is under continuous cattle grazing, permanent overstocking, uncontrolled fires and, in many cases, lacking fencing and with few watering points (Guevara et al. 1997). The herds graze in paddocks, which are then allowed to rest for a short time when foraging reaches its lowest level (Guevara et al. 1993, Gonnet 1998). Cattle density in the paddocks is 500 LSU 5000 ha (Livestock Unit, the grazing equivalent of one adult dairy cow). Other related activities are conducted in the area, such as firewood extraction, subsistence hunting, fencing, roads and human settlements. Grazing hotspots (e.g., ranch centres, water resources) have some woody vegetation grown from cattle-dispersed seeds of *P. flexuosa*, associated with bare soil and grasses (Campos & Ojeda 1997, Asner et al. 2003, Tabeni & Ojeda 2005). Density of adult *P. flexuosa* trees was estimated to be 81 trees ha^{-1} (Aschero & Vázquez 2009).

In the central Monte, the fruit production of *P. flexuosa* is highly variable from year to year; it can be extremely abundant or almost non-existent. All seeds produced are quickly dispersed or consumed by wild and domestic animals (Campos & Ojeda 1997, Villagra et al. 2002, Campos et al. 2007). Because of the harsh climate conditions, plant establishment occurs sporadically. Villagra et al. (2004) proposes that establishment of *P. flexuosa* occurs in pulses and not continuously, generating a coetaneous forest, and not showing a regular distribution of individuals among age classes.

Study design

We developed a design based on two approaches to accomplish our goals. To meet goals 1, 2 and 3 we used an observational approach ("mensurative experiments"; sensu Hurlbert 1984), while for goal 4 we applied an experimental approach ("manipulative experiment"; sensu Hurlbert 1984). For the observational study, we worked in three similar areas, each of them used to reach each observational goal. Areas were located at least 500 m away from each other. Each area consisted of a pair of 25-ha plots (replicates) spaced 2 km apart (Fig. 1). For the experimental study, we worked in one 25-ha area (experimental area) located 500 m away from the observational areas (Fig. 1).

In each plot and in the experimental area, we defined five different cattle-use site classes. Cattle "trails" connect grazing, resting and watering areas; the animals tend to move in a single file along the route of least resistance (Walker & Heitschmidt 1986). Parallel to cattle trails, and extending up to 2 m on either side, we defined "periphery of trails", with presence of fresh dung and cattle eventually destroying the vegetation. "Under *P. flexuosa*" are cattle grazing and resting sites beneath the cover of *P. flexuosa* trees. These sites provide shade for cattle in summertime (Guevara et al. 1997). "Under shrubs" and "bare soil" are sites where isolated individuals only walk; the former sites are under cover of shrubs such as *Larrea* spp., whereas the latter have no plant cover. In each plot and in the experimental area, sampling units were always quadrats (2 m x 1 m) placed at random in all five different cattle-use site classes ("trails", "periphery of trails", "under *P. flexuosa*", "under shrubs" and "bare soil") with a distance of at least 20 m between them.

To meet goals 1, 2 and 3 in the observational approach we measured, on each cattle-use site class: number of seeds m^{-2} transported by cattle dung (seed input; goal 1); number of seedlings m^{-2} 10 months after fruit production and dung deposition when the first rainfall occurred (goal 2); number of established saplings m^{-2} (small plants more than one year old; goal 3). These variables described, in the long term, the contribution of seeds by cattle dispersal and the differences among cattle-use sites. To meet goal 4 in the experimental approach, we quantified during two months, for each cattle-use site class, the number of germinated seeds and remaining seeds (seeds not removed by animals like rodents, beetles and ants, or buried by hoof action) and seedling survival in simulated dung with a known number of seeds. These variables represent, in the short term, the fate of seeds dispersed by cattle on each cattle-use site class.

Observational approach: Seed input via cattle dung

In the first study area (Area 1), we quantified the number of dung-borne seeds in 30 sampling units (quadrats of 2 m x 1 m) placed at random on each cattle-use site class (Fig. 1). To determine whether there was any difference in number of seeds in the dung taken from the different sites, we counted the number of seeds in 15 samples of 100 g of dry weight of dung collected from every site. As we found no significant differences in seed number (± SE) among dung pads from the different sites (208.9 ± 29.4; $F_{(4, 44)} = 1.38$; $P = 0.2609$), we estimated mean number of seeds per 100 g of dry weight of dung. Afterwards, relative cover of dung at each site was estimated in relation to a dung pad 30 x 20 cm in size and 600 g in dry weight. We calculated dung dry weight on the basis of dung coverage in the quadrats for each cattle-use site class, and estimated the number of seeds m^{-2} (seed input via cattle dung) on the basis of number of seeds 100 g^{-1} of dry weight of dung (209 seeds).

Observational approach: Seedlings 10 months after P. flexuosa *fruit production*

In December 2006, once the dry season had ended and after the first rainfalls (50.5 mm in two weeks), the cattle were temporarily removed from the study area and we were able to quantify the number of emerged

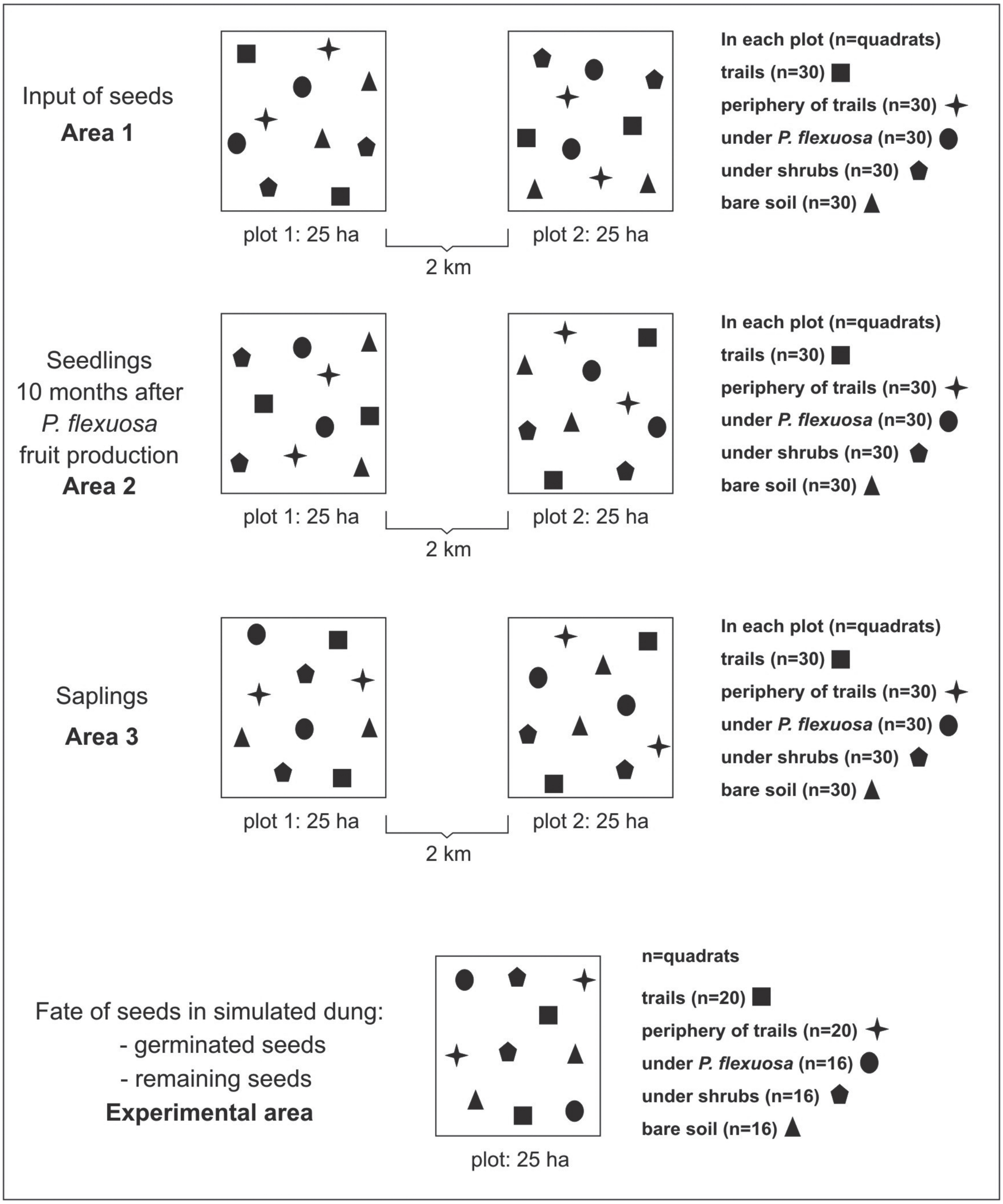

Fig. 1: Design showing the response variables (input of seeds, seedlings 10 months after dung deposition, saplings, germinated seeds and remaining seeds) recorded in the study areas, and the number of quadrants placed at random on each plot in different cattle-use site classes.

Diseño donde se muestran las variables respuesta (aporte de semillas, plántulas 10 meses después de la deposición, renovales, semillas germinadas en los excrementos y semillas que permanecen en los excrementos) registradas en las áreas de estudio y el número de cuadrados localizados aleatoriamente en cada parcela en los diferentes sitios de actividad del ganado.

seedlings. The first rains after the dry season stimulated germination of the seeds lying in the soil, potentially coming from seeds dispersed via dung all year round. Although the seed source could not be determined because dung had lost integrity 10 months after deposition, we know the seed input via cattle dung in the different cattle-use site classes.

In each plot of Area 2 (Fig. 1) we counted the number of seedlings in 30 quadrats placed at random, within each of the cattle-use site classes. Seedling data were not related to the simulated dung or its included seeds, because we worked in a different area.

Observational approach: Saplings

In order to compare the number of established small plants more than one year old (saplings) among the different cattle-use site classes, we worked in a third area (Area 3), different from the experimental area with "seeds in simulated dung" and from the areas where we quantified "seed input" and "seeds 10 months after fruit production" (Fig. 1). We quantified the number of saplings (small established plants, between 10 cm and 150 cm in height) in 30 quadrats randomly placed at each cattle-use site.

Experimental approach: Fate of seeds in simulated dung immediately after P. flexuosa *fruit production*

In February 2006, at the time of *P. flexuosa* fruit production and cattle seed dispersal, we placed simulated dung with a known number of seeds on all cattle-use site classes in the experimental area (Fig. 1), in order to quantify the number of germinated seeds and remaining seeds (seeds not removed by animals like rodents, beetles and ants, or buried by hoof action). Manure and 30 free seeds (seeds with no endocarps; which is the state in which seeds are most commonly found in cattle dung; Campos & Ojeda 1997) extracted from other dung pads were mixed in a plastic pot and, by inverting the pot on the ground, a dung pad approximately 4 cm deep and 14 cm in diameter was formed. One simulated dung pad was placed per quadrat: 20 on "trails" (600 seeds in total), 20 on the "periphery of trails" (600 seeds), 16 at the "under *P. flexuosa*" site (480 seeds), 16 at the "under shrubs" site (480 seeds), and 16 at the "bare soil" site (480 seeds). Each simulated dung pad was marked using a flag tied to the nearest bush.

Number of germinated seeds in the dung and dung condition (trampled or untrampled) were monitored every seven days for two months (from February to April). Over this time, rainfalls totalled 99 mm. Seedlings were marked and monitored to determine survival during two months. At the end of the experiment, we retrieved the untrampled simulated dung pads and counted the number of seeds persisting in them. Some simulated dung pads had been totally disintegrated by trampling and we could not find any seeds in them. Seeds missing from untrampled dung had been removed by animals (rodents, insects, and birds), but in trampled dung these seeds could have been either buried by trampling or removed by animals. For this reason, we can clearly discriminate only between two fates: germinated seeds and seeds remaining in the dung.

Statistical analysis

Analyses were conducted with R statistical software version 2.11.1 (R Development Core Team 2010, http://www.r-project.org/). To assess whether the explanatory variable "cattle-use site classes" ("trails", "periphery of trails", "under *P. flexuosa*", "under shrubs" and "bare soil") affected the response variables ("seeds m^{-2} transported by cattle dung ", "number of seedlings m^{-2} 10 months after fruit production and dung deposition when the first rainfall occurred", and "number of established saplings/m^{-2}") we fitted generalized linear mixed models (GLMM), with cattle-use site classes as a fixed factor with five levels and plots as a random factor with two levels. A Poisson error distribution with a log-link function was applied, using the lmer function of R's lme4 package. The significance of the fixed factor was assessed using Wald statistics test (Bolker et al. 2008).

In order to compare the fate of seeds from simulated dung among the different cattle-use site classes, as germinated seeds were not enough in number, we used the number of remaining seeds. We performed a generalized linear model (GLM) using the explanatory variable "cattle-use site classes" and the response variable "remaining seeds". We assumed a Poisson error distribution and used a log-link function (McCullagh & Nelder 1989). The significance of the fixed effect was assessed using Likelihood Type 1 Test due to the high frequency of zeros in the response variable.

RESULTS

Mixed effects modelling suggested strong effects of cattle-use sites on input of dung-borne seeds (GLMM: χ^2 = 2535.2; df = 1; P = 0.001). The highest seed input occurred at the "under *Prosopis*" site and "periphery of trails". The seed input was lower on "trails", "bare soil" and "under shrubs" (Fig. 2A).

Ten months after fruit production and deposition of dung with seeds, we found 3,637 just emerged seedlings. The number of seedlings was affected by cattle-use sites (GLMM: χ^2 = 203.5; df = 1; P = 0.001). The highest number of seedlings occurred on "trails", followed by "under *Prosopis*" and "periphery of trails", while presence of seedlings "under shrubs" and on "bare soil" was almost non-existent (Fig. 2B).

The number of established saplings was affected by cattle-use sites (GLMM: χ^2 = 6.8; df = 1; P = 0.01). The highest number of saplings occurred in "periphery of trails", followed by "under *Prosopis*", "trails", "under shrubs", and "bare soil" (Fig. 2C).

The number of germinated seeds in simulated dung immediately after fruit production and dung deposition was very low, only 0.19 % (five seeds) of a total of 2640 seeds germinated across all cattle-use site classes (0.4 % "under *Prosopis*", the highest

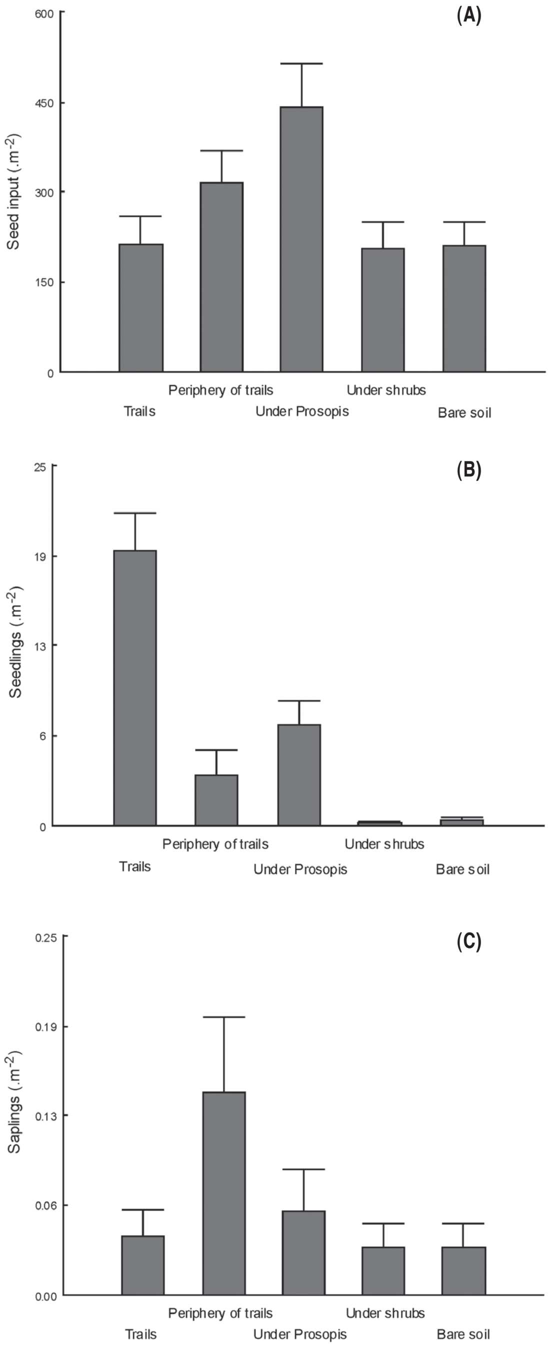

Fig. 2: Mean (± SE) number of seeds m^{-2} transported in cattle dung (A), number of seedlings m^{-2} 10 months after fruit production and dung deposition when the first rainfall occurred (B), and number of established saplings m^{-2} (C), on different cattle-use site classes.

Número promedio (± EE) de semillas m^{-2} transportadas por el ganado (A), de plántulas m^{-2} 10 meses después de la fructificación y la deposición de los excrementos con semillas, cuando ocurren las primeras lluvias (B) y de renovales establecidos m^{-2} (C) en los diferentes sitios de actividad del ganado.

frequency). None of the seedlings survived for more than one week. The number of remaining seeds (seeds not buried or removed by animals, such as rodents, insects, and birds) in intact simulated dung (with an initial number of 30 seeds) was affected by cattle-use site classes (GLM: $\chi^2 = 307.4$; df = 1; P = 0.001). On "trails" we found no remaining seeds in the dung, which was all trampled. The lowest number of remaining seeds occurred on "periphery of trails", where only 4 % (mean ± SE, 1.15 ± 0.57, n = 20) of the seeds persisted in intact dung. The highest number of remaining seeds occurred "under shrubs", "under *Prosopis*", and on "bare soil", with 27.5 % (8.25 ± 1.66, n = 16), 21.7 % (6.5 ± 1.56, n = 16), and 20.8 % (6.25 ± 2.09, n = 16) of remaining seeds, respectively.

DISCUSSION

In the present study, a high number of seeds was found per 100 g of dung (mean = 208.9), compared to a former study conducted in the same area where the total number of seeds encountered was 77.5 (Campos & Ojeda 1997). The differences found may be the result of the high annual variability in pod production by *P. flexuosa* trees in the Monte desert (Villagra et al. 2004). Space use by cattle produced differential seed input into the different cattle-use site classes. "Under *Prosopis*" and in the "periphery of trails" animals deposited the largest amounts of dung and seed. Ten months after dung deposition, seeds germinated immediately after the end of winter, when the first rains begin to fall. The highest number of seedlings occurred on "trails", "under *Prosopis*" and in the "periphery of trails", where seeds somehow returned to the soil, probably because of trampling activity. In the long term, the highest number of established saplings occurred only in the "periphery or trails".

The number of germinated seeds in simulated dung immediately after fruit production and dung deposition was very low. Survival of seedlings emerging from seeds germinated in dung did not exceed one week. Seed remaining in dung varied in the different cattle-use site classes: on "trails" and in the "periphery of trails" the persistence of seeds in dung was low because of dung disintegration by cattle trampling. The seeds that did not remain in dung were probably the source of seeds that will germinate in the next wet season (i.e. 10 months after dung deposition). As observed for other endozoochorous interactions, spatial patterns of deposition by herbivores, activities associated with grazing (trampling, urination, etc.), avoidance of or attraction to dung-borne seeds by predators and secondary dispersers, dung degradation process and climate conditions, among others, are factors that affect seed input into the soil, seed germination and seedling survival and establishment (Peinetti et al. 1992, Malo & Suárez 1995a, Malo et al. 2000).

As in other studies that have shown differences in spatial patterns of defecation by herbivores and seed deposition, linked to land use at small and medium scales (Malo et al. 2000), in this study we found that the different cattle-use site classes exhibited a different input of seed. At small scale, the daily use pattern may explain the differences in seed input. Resting and foraging sites for cattle ("under *Prosopis*") and sites related to movement of animals ("periphery of trails") were the sites with greater seed input, whereas sites with heavy traffic ("trails") or low general activity ("under shrubs" and "bare soil") received smaller amounts of seed.

In the present study, the effect of seed transport and of activities resulting in dung disintegration (such as trampling) appears to be important, because the highest number of seedlings 10 months after dung deposition occurred at sites with heavier cattle traffic ("trails") and at sites with greater seed input ("under *Prosopis*" and "periphery of trails"). At heavily travelled cattle-use sites, trampling has a major effect because it involves dung disintegration, separation of seeds from faecal material and seed burial. Probably, in the case of *Prosopis* seeds that need previous scarification to germinate (Solbrig & Cantino 1975), trampling can help by producing breakage of the coat by friction with the loose substrate particles removed.

Non-germinating seeds that remained in intact dung may disappear, which does not necessarily indicate seed predation by animals but may simply indicate that a new stage in the seed dispersal process has begun (Vander Wall et al. 2005). Seed removal by beetles, ants and rodents is considered to be an example of diplochory (Vander Wall & Longland 2004, 2005). In this manner, secondary dispersers may increase the probability of seed survival because seeds in dung are often attacked by fungi and microbes, they are susceptible to desiccation, and seedling competition is higher (Wenny 1999). Although in this study we cannot discriminate which animals removed seeds, small rodents and ants in the area are known to remove them from faeces of *Dolichotis patagonum* (Villagra et al. 2002) and from cattle dung (S Velez & C Campos, unpublished data). We also observed beetles removing faecal material from inside the dung, leaving only the hardened outer layer intact.

In the long term, the highest number of established saplings occurred only in the "periphery of trails". Although the number of seedlings was high on "trails", "under *Prosopis*"and in the "periphery of trails", continuous trampling on "trails" and the likely inappropriate conditions for seedling establishment "under *Prosopis*" prove the "periphery of trails" to be the site with highest occurrence of saplings. Augspurger (1984) reports a higher number of saplings in areas with removed vegetation and increased light level. Particularly the saplings of *Prosopis* spp. occupy bare, arid microsites far from tree and shrub canopies, and with high percentages of incident light (Páez & Marco 2000), because solar radiation availability improves seedling growth and development (Vilela & Ravetta 2000). *Prosopis* species colonize open patches, taking advantage of the low competition, and become established during the brief favourable period before the dry season. In semiarid areas, these species are regarded as dominant and can be important nurse species in these ecosystems (Páez & Marco 2000, Rosi & Villagra 2003).

Finally, our results showed a very low percentage of seed germination within 2 months after dung deposition. Only 0.19 % of the total seeds (2640) germinated. Dung characteristics such as toxicity for seedlings, low affinity for water and lower level of light penetration (Lobo & Veiga 1990, Cosyns et al. 2006) may result in low percentages of seed germination in dung (Malo & Suárez 1995a, 1995b). These results match those reported for semiarid grasslands, where germination of some species is not high after dung deposition, although seed input produces an increased number of plants after the following rains and in the succeeding years (Malo & Suárez 1995b, Cosyns et al. 2006). The higher number of seedlings at sites with deposition of herbivore excrements has been attributed to endozoochory, to gap creation and to nutrient enrichment produced by dung decomposition (Dai 2000, Cosyns et al. 2006).

The role of cattle in *P. flexuosa* seed dispersal is not limited to the effect of gut passage on seed viability and germination. Cattle transport a huge amount of seeds in a spatially differential manner and tramping activity appears to have an important effect. Although only few seeds germinate immediately after defecation, and none of the seedlings survive for more than one week, seeds contained in dung can return to the soil after dung decomposition and trampling by cattle. Probably trampling helps seed scarification, because a large quantity of seeds can germinate in the following wet season at the site more heavily trodden by cattle ("trails"). But continuous trampling hampers sapling establishment, because, in the long term, the best site for sapling establishment is the "periphery of trails", where the plants have good sun exposure and lower trampling activity than on "trails".

In conclusion, with different effects depending on cattle site activity and the stage of the *P. flexuosa* plant (seed, seedling, or sapling), defecation and trampling appear to be important processes in the seed dispersal cycle. In this sense, cattle could benefit the establishment of *P. flexuosa*. In a similar ecosystem but within the Man and Biosphere Reserve of Ñacuñán, with no cattle since 1970 (Ojeda et al. 1998), experiments with permanent plots indicate that seedling survival and sapling establishment have been almost non-existent over the past 10 years (Villagra et al. 2004). The latter study supports the importance of cattle in the seed dispersal cycle in this arid region, in keeping with our findings in the present study.

ACKNOWLEDGEMENTS: We thank Cecilia Bertolini and Genny Trovato for their help with the field work. Nelly Horak for assisting us with the English version. Natalia Andino helped us with statistical analyses. We appreciate the comments and suggestions given by the editor and anonymous reviewers. Research was funded by CONICET (PIP 5940).

LITERATURE CITED

ANDRESEN E & F FEER (2005) The role of dung beetles as secondary seed dispersers and their effect on plant regeneration in tropical rainforests. In: Forget P-M, JE Lambert, PE Hulme & SB Vander-Wall (eds) Seed fate: Predation, dispersal and seedling establishment: 331-349. CAB International, UK.

ASCHERO V & DP VÁZQUEZ (2009) Habitat protection, cattle grazing and density-dependent reproduction in a desert tree. Austral Ecology 34: 901-907.

ASNER GP, CE BORGHI & RA OJEDA (2003) Desertification in central Argentina: Changes in ecosystem carbon and nitrogen from imaging spectroscopy. Ecological Applications 13: 629-648.

AUGSPURGER CK (1984) Light requirements of neotropical tree seedlings: A comparative study of growth and survival. Journal of Ecology 72: 777-795.

BOLKER BM, ME BROOKS, CJ CLARK, SW GEANGE, JR POULSEN, MHH STEVENS & JSS WHITE (2008) Generalized linear mixed models: A practical guide for ecology and evolution. Trends in Ecology and Evolution 24: 127-135.

BROWN JR & S ARCHER (1987) Woody plant seed dispersal and gap formation in a North American subtropical savanna woodland: The role of domestic herbivores. Vegetatio 73: 73-80.

BROWN JR & S ARCHER (1989) Woody plant invasion of grasslands: Establishment of honey mesquite (*Prosopis glandulosa* var. *glandulosa*) on sites differing in herbaceous biomass and grazing history. Oecologia 80: 19-26.

CAMPOS C & R OJEDA (1997) Dispersal and germination of *Prosopis flexuosa* (Fabaceae) seeds by desert mammals in Argentina. Journal of Arid Environments 35: 707-714.

CAMPOS CM, SM GIANNONI, P TARABORELLI & CE BORGHI (2007) Removal of mesquite seeds by small rodents in the Monte desert, Argentina. Journal of Arid Environments 69: 228-236.

CAMPOS CM, B PECO, VE CAMPOS, JE MALO, SM GIANNONI & F SUÁREZ (2008) Endozoochory by native and exotic herbivores in dry areas: Consequences for germination and survival of *Prosopis* seeds. Seed Science Research 18: 91-100.

CLARK JS, B BECKAGE, P CAMILL, B CLEVELAND, J HILLERISLAMBERS et al. (1999) Interpreting recruitment limitation in forest. American Journal of Botany 86: 1-16.

COSYNS E, B BOSSUYT, M HOFFMANN, H VERVAET & L LENS (2006) Seedling establishment after endozoochory in disturbed and undisturbed grasslands. Basic Applied Ecology 7: 360-369.

DAI X (2000) Impact of cattle dung deposition on the distribution pattern of plant species in an alvar limestone grassland. Journal of Vegetation Science 11: 715-724.

EILBERG BA (1973) Presencia de disemínulos de "vinal" (*Prosopis ruscifolia* Griseb) en deyecciones de equinos y bovinos. Ecología 1: 56-57.

ESTRELLA H, J BOSHOVEN & M TOGNELLI (2001) Características del clima regional y de la Reserva de Ñacuñán. In: Claver S & S Roig-Juñent (eds) El desierto del Monte: La Reserva de Biosfera de Ñacuñán. Mendoza, Argentina: 25-33. Editorial Triunfar, Córdoba, Argentina.

FENNER M & K THOMPSON (2005) The ecology of seeds. University Press, Cambridge.

FISHER CE (1977) Mesquite and modern man in south-western North America. In: Simpson BB (ed) Mesquite: Its biology in two desert ecosystems: 177-187. Dowden, Hutchinson & Ross Inc, Pennsylvania.

GONNET JM (1998) Impacto del pastoreo sobre poblaciones de aves y mamíferos hervíboros en la región de la Reserva de la Biósfera Ñacuñán, Mendoza, Argentina. Doctoral Thesis, Universidad Nacional de Córdoba, Córdoba, Argentina.

GRACE JB & D TILMAN (1990) Perspectives on plant competition. Academic Press, USA.

GUEVARA JC, JA PÁEZ & OR ESTÉVEZ (1993) Caracterización económica de los principales sistemas de producción ganadera en el árido mendocino. Multequina 2: 259-273.

GUEVARA JC, B CAVAGNARO, OR ESTÉVEZ, HN LE HOUÉROU & CR STASI (1997) Productivity, management and development problems in the arid rangelands of the central Mendoza plains (Argentina). Journal of Arid Environments 35: 575-600.

HARPER JL (1977) Population biology of plants. Academic Press, London.

HOWE HF & J SMALLWOOD (1982) Ecology of seed dispersal. Annual Review of Ecology and Systematics 13: 201-228.

HURLBERT SH (1984) Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54: 187-211.

JANZEN DH (1970) Herbivores and the number of tree species in tropical forests. American Naturalist 104: 501-528.

JANZEN DH (1982) Removal of seeds from horse dung by tropical rodents: Influence of habitat and amount of dung. Ecology 63: 1887-1900.

JANZEN DH (1983) Seed and pollen dispersal by animals: Convergence in the ecology of contamination and sloppy harvest. Biological Journal of the Linnean Society 20: 103-113.

JANZEN DH (1986) Mice, big mammals and seeds: It matters who defecates what where. In: Estrada A & TH Fleming (eds) Frugivores and seed dispersal: 314-338. Dr. W. Junk Publishers, The Netherlands.

KITAJIMA K & M FENNER (2000) Ecology of seedling regeneration. In: Fenner M (ed) Seeds: The ecology of regeneration in plant communities: 331-360. Commonwealth Agricultural Bureau International, Wallingford, UK.

KINGSOLVER JM, CD JOHNSON, SR SWIER & A TERAN (1977) *Prosopis* fruits as a resource for invertebrates. In: Simpson BB (ed) Mesquite: Its biology in two desert ecosystems: 108-122. Dowden, Hutchinson & Ross Inc, Pennsylvania.

KNEUPER CL, CB SCOTT & WE PINCHAK (2003) Consumption and dispersion of mesquite seeds by ruminants. Journal of Range Management 56: 255-259.

KRAMP BA, RJ ANSLEY & TR TUNNELL (1998) Survival of mesquite seedlings emerging from cattle and wildlife feces in a semi-arid grassland. The Southwestern Naturalist 43: 300-312.

LOBO JM & CM VEIGA (1990) Interés ecológico y económico de la fauna coprófaga en pastos de uso ganadero. Ecología 4: 313-331.

MALO JE & F SUÁREZ (1995a) Establishment of pasture species on cattle dung: The role of endozoochorous seeds. Journal of Vegetation Science 6: 169-174.

MALO JE & F SUÁREZ (1995b) Cattle dung and the fate of *Biserrula pelecinus* L. (Leguminosae) in a Mediterranean pasture: Seed dispersal, germination and recruitment. Botanical Journal of the Linnean Society 118: 139-148.

MALO JE, B JIMÉNEZ & F SUÁREZ (2000) Herbivore dunging and endozoochorous seed deposition in a Mediterranean dehesa. Journal of Range Management 53: 322-328.

MCCULLAGH P & JA NELDER (1989) Generalized linear models. Chapman and Hall, London.

MOONEY HA, BB SIMPSON BB & OT SOLBRIG (1977) Phenology, morphology, physiology. In: Simpson BB (ed) Mesquite: Its biology in two desert ecosystems: 26-45. Dowden, Hutchinson & Ross Inc, Pennsylvania.

MORELLO J (1958) La provincia fitogeográfica del Monte. Opera Lilloana 2: 1-155.

OJEDA R, C CAMPOS, J GONNET, C BORGHI & V ROIG (1998) The MaB Reserve of Ñacuñan, Argentina: Its role in understanding the Monte desert biome. Journal of Arid Environments 39: 299-313.

OLFF H & M RITCHIE (1998) Effects of herbivores on grassland plant diversity. Trends in Ecology and Evolution 13: 261-265.

ORTEGA-BAES P, M VIANA & S SUHRING (2002) Germination in *Prosopis ferox*: Effects of mechanical, chemical and biological scarificators. Journal of Arid Environments 50: 185-189.

PÁEZ SA & DE MARCO (2000) Seedling habitat structure in dry Chaco forest (Argentina). Journal of Arid Environments 46: 57-68.

PEINETTI R, C CABEZAS, M PEREYRA & O MARTÍNEZ (1992) Observaciones preliminares sobre la diseminación del caldén (*Prosopis caldenia* Burk). Turrialba (Costa Rica) 42: 415-417.

PEINETTI R, M PEREYRA, A KIN & A SOSA (1993) Effects of cattle ingestion on viability and germination rate of caldén (*Prosopis caldenia*) seeds. Journal of Range Management 46: 483-486.

R DEVELOPMENT CORE TEAM (2010) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/ (accessed December 10, 2010).

ROIG FA (1971) Flora y vegetación de la Reserva Forestal de Ñacuñán. Deserta (Argentina) 1: 25-232.

ROSSI BE & PE VILLAGRA (2003) *Prosopis flexuosa* effects on the spatial pattern of understory species and soil properties in the Monte desert (Argentina). Journal of Vegetation Science 14: 543-550.

SOLBRIG OT & PD CANTINO (1975) Reproductive adaptations in *Prosopis* (Leguminosae,

Mimosoideae). Journal of the Arnold Arboretum (UK) 56: 185-210.
STONER KE, P RIBA-HERNÁNDEZ, K VULINEC & JE LAMBERT (2007) The role of mammals in creating and modifying seed shadows in tropical forests and some possible consequences of their elimination. Biotropica 39: 316-327.
TABENI S & RA OJEDA (2005) Ecology of the Monte Desert small mammals in disturbed and undisturbed habitats. Journal of Arid Environments 63: 244-255.
TANQUILEVICH RF (1971) Los suelos de la Reserva Ecológica de Ñacuñán. Deserta (Argentina) 2: 131-206.
VANDER WALL SB & WS LONGLAND (2004) Diplochory: Are two seed dispersers better than one? Trends in Ecology and Evolution 19: 155-161.
VANDER WALL SB, P-M FORGET, JE LAMBERT & PE HULME (2005) Seed fate pathways: Filling the gap between parent and offspring. In: Forget P-M, JE Lambert, PE Hulme & SB Vander Wall (eds) Seed fate: Predation, dispersal and seedling establishment: 1-8. CAB International, London.
VANDER WALL SB & WS LONGLAND (2005) Diplochory and the evolution of seed dispersal. In: Forget P-M, JE Lambert, PE Hulme & SB Vander Wall (eds) Seed fate: Predation, dispersal and seedling establishment: 297-314. CAB International, London.
VILELA AE & DA RAVETTA (2000) The effect of radiation on seedling growth and physiology in four species of *Prosopis* L. (Mimosaceae). Journal of Arid Environments 44: 415-423.
VILLAGRA PE, L MARONE & M CONY (2002) Mechanisms affecting the fate of *Prosopis flexuosa* (Fabaceae, Mimosoideae) seeds during early secondary dispersal in the Monte Desert, Argentina. Austral Ecology 27: 416-421.
VILLAGRA PE, MA CONY, NG MANTOVÁN, BE ROSSI, M GONZÁLEZ-LOYARTE, R VILLALBA & L MARONE (2004) Ecología y manejo de los algarrobales de la provincia fitogeográfica del Monte. In: Arturi MF, JL Frangi & JF Goya (eds) Ecología y manejo de bosques nativos de Argentina: 2-32. Universidad Nacional de La Plata, La Plata, Argentina.
WALKER JW & RK HEITSCHMIDT (1986) Effect of various grazing systems on type and density of cattle trails. Journal of Range Management 39: 428-431.
WANG BC & TB SMITH (2002) Closing the seed dispersal loop. Trends in Ecology and Evolution 17: 379-385.
WENNY DG (1999) Two-stage dispersal of *Guarea glabra* and *G. kunthiana* (Meliaceae) in Monteverde, Costa Rica. Journal of Tropical Ecology 15: 481-496.

Associate Editor: Ernesto Gianoli
Received October 6, 2010; accepted May 11, 2011