

Diplochory in two *Jatropha* (Euphorbiaceae) species of the Monte Desert of Argentina

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Abstract The potential explosive seed dispersal under controlled conditions and the dispersal by ants in natural populations are compared between two diplochoric species: *Jatropha hieronymi* Kuntze and *J. excisa* Griseb. The seeds of *J. hieronymi* are more than eightfold heavier than *J. excisa* seeds, and were explosively dispersed considerably further distances, reaching a maximum of almost 18 m. The differences in explosive dispersal distances between the two species seem to depend on both carpel wall thickness of the fruit and aerodynamic shape of the seed. Seed removal by ants was positively correlated with the presence of the elaiosome and was higher for *J. excisa* (83.6%) than for *J. hieronymi* (31.6%). Seed size was the major factor affecting the removal by ants, as only large bodied ants were able to transport the large seeds of *J. hieronymi*. The larger size and the higher oleic acid content of the elaiosomes of *J. hieronymi* seeds had no influence on the observed removal rates by ants. In contrast, ants transported the *J. hieronymi* seeds further distances than *J. excisa* seeds. *Jatropha hieronymi* distances achieved by both dispersal modes are in the range of the furthest distances described for a diplochorous species. Finally, the possible advantages of this dispersal mode in arid zones are discussed.

Key words: arid zone, diplochory, *Jatropha*, dispersal distances, seed dispersal.

INTRODUCTION

Diplochory is seed dispersal by a sequence of two or more steps, each involving a different dispersal agent. The combination of two dispersal mechanisms often provides greater benefits to seeds than do most single means of dispersal (Vander Wall & Longland 2004). For plants with such mechanism, phase one dispersal moves seeds away from the influence of the parent plant (reducing parent and sibling competition) and phase two often moves seeds to safe sites, where the chance of successful germination and seedling establishment are enhanced.

Diplochory is frequent in the Euphorbiaceae family (Webster 1994). Seeds are initially dispersed using ballistic dispersal, where the seeds are discharged by the explosive dehiscence of the fruit produced by the elastic contraction of its tissues (Garrison *et al.* 2000). Seeds are subsequently dispersed by myrmecochory, dispersal of seeds by ants, which are attracted to a seed appendage termed the elaiosome. The elaiosome on the seeds of Euphorbiaceae dispersed by ants is formed by a lipid-rich caruncle (Lisci *et al.* 1996). The elaiosome frequently acts as a nutritional reward for ants (Bresinsky 1963; Beattie 1985; Morales & Heithaus 1998; Wolff & Debussche 1999) and at least in some species, its composition mimics that of insect

prey (Hughes *et al.* 1994a). Ants collect the elaiosome-bearing seeds and take them to their nests, where the elaiosome is consumed by workers and/or larvae and the seed is then left in a chamber underground (Culver & Beattie 1980) or ejected from the nest as waste (Handel 1976; Horvitz & Beattie 1980; O'Dowd & Hay 1980; Bullock 1989; Harrington and Driver 1995).

Various studies have evaluated the relative importance of explosive and myrmecochorous dispersal in diplochorous species (Culver & Beattie 1980; Stamp & Lucas 1983; Ohkawara & Higashi 1994; Lisci & Pacini 1997; Narbona *et al.* 2005). However, the relationships between the two dispersal modes and the hypothesized benefits for plant establishment deriving from the combination of both dispersal mechanisms are not clear. In particular, dispersal by explosive dehiscence has few previous quantified data. It has been reported that the distance to which the ants move the seeds is greater than that achieved by explosive dispersal (Stamp & Lucas 1990). The presence of an elaiosome for myrmecochorous dispersal may alter the seed aerodynamics, reducing the distance of explosive dispersal (Beattie & Lyons 1975). Because of constraints of the explosive mechanism, plant species that use explosive dispersal are postulated to maximize either explosive distances or secondary dispersal (Beattie & Lyons 1975; Stamp & Lucas 1983).

Diplochory by explosive dehiscence followed by dispersal by ants has been reported for many

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Euphorbiaceae native to the Northern Hemisphere (Lisci *et al.* 1996; Narbona *et al.* 2005), but only a few studies are available for euphorbs native to South America, and these were carried out in the Caatinga Region of North-east Brazil (Leal *et al.* 2007). Here, we evaluate diplochory by comparing two *Jatropha* (Euphorbiaceae) species native to the Monte Desert of North-west Argentina. This semi-arid region has ecological characteristics similar to other areas where myrmecochory is a dominant dispersal syndrome, such as the Mediterranean scrublands of Australia (Hughes & Westoby 1992) and the South African Fynbos (Milewski & Bond 1982). The two species were chosen as they differ in seed and fruit morphological features that may influence both dispersal modes. Seeds of both species are primarily explosively dispersed and have a conspicuous elaiosome.

The objectives of this study were to determine: (i) the explosive dispersal distances of *Jatropha excisa* and *J. hieronymi* seeds under experimental conditions and the influence of seed and fruit morphological features on these distances; and (ii) the secondary dispersal of the two *Jatropha* species by ants in the field and the influence of the elaiosome size, seed size and fatty acid composition of the elaiosomes on the number of seeds removed and the dispersal distances.

MATERIAL AND METHODS

Study sites and species

Jatropha hieronymi Kuntze occurs in the north-west pre-Andean regions of Argentina and Bolivia; *Jatropha excisa* Griseb is commonly found in north-west and central Argentina and Paraguay (Lourteig & O'Donnell 1943). The collection sites of this study were located in the La Rioja province of Argentina; *J. hieronymi* was collected in Juan Caro, 10 km north of the city of La Rioja (29°14'S, 66°48'W) whereas *J. excisa* was collected 30 km north-east of the town of Anillaco (28°54'S, 66°39'W). Altitudes from both sites range from 700 to 800 m a.s.l. The climate is semi-arid to arid (Morello 1958), with a mean annual rainfall of approximately 300 mm confined to the wet summer season and a mean annual temperature of 16.6°C. The Monte Desert (*sensu* Cabrera 1976) in the La Rioja province is an open shrubland dominated by perennial aphyllous and sub-aphyllous shrubs species of the families Zygophyllaceae (*Larrea cuneifolia* and *Bulnesia retama*), Fabaceae (*Senna aphylla* and *Cercidium praecox*) and Cactaceae (*Trichocereus* sp., *Tephrocactus* sp. and *Opuntia* sp.). The two *Jatropha* species constitute one-species patches but also mixed patches, with *J. excisa* being the more widespread and more capable of growing at higher altitudes (A. Rickert pers. obs. 2008).

Jatropha hieronymi and *J. excisa* are perennial and deciduous shrubs, reaching heights up to 3 and 1.5 m, respectively, at our study sites, with a canopy diameter <2 m. The inflorescences are monoecious and bloom during the summer,

fruits dehisc from the end of the summer to the beginning of the autumn (from February to April) and seed dispersion takes place at the beginning of the dry period. Fruits are tricarpetate capsules with each carpel containing one seed. Seeds are carunculate, and the caruncle always remains attached to the seed body after the seeds are explosively discharged (A. Rickert pers. obs. 2008). Drying of the fruit leads to dehiscence, where each woody carpel separate from the central axis, splitting into two valves and explosively dispersing the seeds. Capsules of *J. hieronymi* are pendulous during dispersal, whereas *J. excisa* fruit stalks are sub-erect at the moment of explosion (Fig. 1C,D). Preliminary studies carried out in our laboratory have shown that seeds of both species are dormant for at least the winter period.

Collection of seeds

Almost ripe fruits of the two species were collected from the study sites described above between February and April 2008. Fruits were randomly sampled from 20 different individuals of each population. Fruits were left to discharge inside plastic containers exposed to the sun, and the newly collected seeds were used for all seed measurements. Empty seeds (without embryo) were recognized because they broke down easily when pressed between the fingers, and were consequently discarded. For the ant removal experiments, seeds discharged the day before were used so that the elaiosome was fresh.

Seed and fruit morphology

Seed mass, length and width were determined for 80 seeds for each species. Seeds were weighed with an electronic balance. Elaiosome was obtained by removing it from the seed body with a sharp blade and then weighed separately. Fruit dimensions were determined for 30 mature and dry fruits. The mechanical layer of the fruit walls was studied using sections of dry carpels stained with phloroglucinol 10% (Harris *et al.* 1994) in order to identify the presence of lignin in the cell walls. Sections were analysed by light microscopy.

Fatty acid composition of the elaiosomes

Fresh elaiosomes from recently released seeds of *J. hieronymi* and *J. excisa* were analysed for lipid content and fatty acid composition according to the method described by Bligh & Dyer (1959). Elaiosomes were obtained from 120 seeds of *J. hieronymi* and 200 seeds of *J. excisa*. Seeds were randomly collected from 20 different plants at the collection sites described above. The fatty-acid methyl esters prepared from the lipid fraction were analysed by gas-liquid chromatography using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector and CP-Wax 52 CB (Chrompack, Holland) capillary column (25 m length; 0.32 mm i.d.; 0.22 µm film thickness) of fused silica. The fatty acids were identified by comparison with retention times of a known standard mixture (AOACS RM-1, Sigma Aldrich, St Louis, MO). Total oil content was determined by weight and expressed as % oil (w/w) per 100 g dry

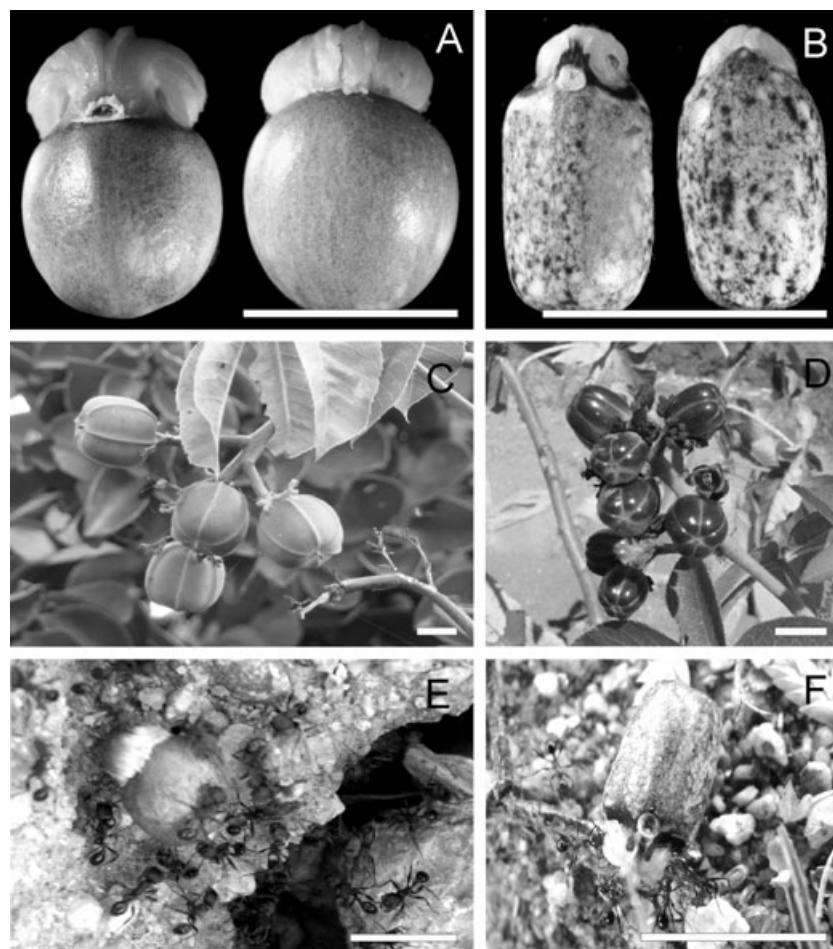


Fig. 1. Seeds, fruits and seed transport by ants of *Jatropha hieronymi* and *J. excisa*. (A) Carunculate seed of *J. hieronymi*. (B) Carunculate seed of *J. excisa*. (C) Unripe capsules with hanging peduncles of *J. hieronymi*. (D) Unripe capsules with sub-erect peduncles of *J. excisa*. (E) *J. hieronymi* seed being dragged by a group of ants into a nest entrance. (F) A group of ants dragging a *J. excisa* seed, holding the elaiosome in their mandibles. Scale bars: 10 mm.

elaiosomes. Fatty acids are expressed as the relative percentage of each individual fatty acid of the total fatty acids present in the sample. All chemical determinations were made in triplicate.

Explosive seed dispersal

Seven stalks bearing fruits of *J. excisa* (7–10 fruits per stalk) and 20 single fruits of *J. hieronymi* were attached to a 4 m long wire hung between two posts at a height of 1.80 m. The orientation of the fruits on the plant was conserved with *J. excisa* stalks being attached erect and *J. hieronymi* stalk capsules procumbent. The system was placed outdoors in the centre of an open flat space at the CRILAR research institute, and the ground covered with canvas sheeting (20 × 20 m) to prevent dispersed seeds from rolling after landing (Narbona *et al.* 2005). None of the two *Jatropha* species grow in the experimental area. The experiment was repeated twice and each trial was conducted for 2 weeks. The experimental area was checked every three hours during the day, from 07.00 to 19.00 hours. When a fruit had dehisced, the distance between

each seed lying on the sheets and the fruit was measured using a measuring tape. When more than one fruit had burst on the same day, the distance to the closest newly dehisced fruit was measured, to avoid overestimating dispersal distances. Then, each newly dispersed seed was weighed separately to find possible relationships between seed weight and dispersal distance. The thickness of wall carpels of the dehisced fruits was also measured. All capsules exploded within 1–4 days from the beginning of the experiment, and were replaced with new capsules in order to reach a suitable observations number. Each trial was always performed under sunny, windless conditions. In total, 226 seeds of *J. hieronymi* and 370 seeds of *J. excisa* were assessed for dispersal distance and weight.

Seed removal and ant-dispersal distances

The experiments of seed removal by ants were carried out in Juan Caro, an undisturbed area where populations of both species are found in the same stand. Fresh seeds from recently opened fruits collected from the sites described above were used for the experiment, as dried elaiosomes may be less attractive to ants.

The seed removal experiment was carried out in order to determine whether ants disperse seeds in the study area and to assess the percentage of seed removal for each plant species. Two 80-m transects 20 m apart were randomly established at the study site. For each transect, we placed mixed groups of five seeds of each species on circular cardboard plates 10 cm in diameter. Each plate was separated by 10 m from the next along each transect, ensuring that ants from different colonies had access to seeds in different samples. Each plate was placed on flat soil surface and firmly anchored to the ground with 6 cm nails. Seeds were set up at 07.00 hours and checked at 2-h intervals, from 07.00 to 19.00 hours. A given seed was considered removed if it was not found within 30-cm of the seed group, and the remaining seeds on each plate were counted at the end of the experiment. In order to assess the distance of seed removal, we followed ants carrying seeds until they entered their nests or disappeared in the leaf litter. The distance of transport was determined with a measuring tape. Observers remained still to minimize the effects on the ant community. Removal experiments were conducted in February, March and April 2008 (i.e. the season when dispersal naturally occurs).

In order to compare differences in the number of seeds removed between seeds with and without elaiosomes, we followed the same procedure described above, but used 160 seeds of each species (half with their elaiosomes detached) placed along two 80 m transects 20 m apart, one transect for each species. On each plate we placed 10 seeds with their elaiosomes, and on the paired plate 10 seeds with their elaiosomes detached. Seeds were set up at 07.00 hours and checked at 2-h intervals, from 07.00 to 19.00 hours. At the end of the experiment we counted the number of seeds with and without elaiosome that had been removed. Experiments were conducted in February, March and April 2008.

Removal by vertebrates

To examine whether vertebrates prey on the *Jatropha* seeds, 10 Petri dishes containing five seeds of each species were randomly placed within the two collection sites described above, approximately 10 m apart from each other. Ants were excluded by application of a non-toxic sticky substance (Tanglefoot) around the edge of the Petri dish. Sites were checked every day for 4 days. This experiment was repeated in February, March and April 2008.

Data analysis

Because the autochoric and myrmecochoric dispersal distances showed highly skewed distributions, all pairwise comparisons were made with non-parametric Mann-Whitney *U*-tests. Simple linear regressions were used to analyse the relationships between seed size and explosive dispersal distance. Differences in the number of seeds removed by ants between both species were analysed with chi-squared tests. A two-way ANOVA was performed to test for the effects of presence/absence of elaiosome and plant species (independent variables) on the number of seeds removed by ants (dependent variables). The dependent variable was log

$(x + 1)$ transformed prior to the analysis to achieve uniformity of normality. Analyses were performed using the STATISTICA 7.0 package (StatSoft Inc. 1998).

RESULTS

Seed and fruit morphology

Seeds of *J. hieronymi* and *J. excisa* substantially differed in their morphological features. *Jatropha hieronymi* seeds are considerably larger and heavier than *J. excisa* seeds (Table 1). Figure 1A and B shows the differences in seed shape between the two species and the prominent elaiosome of *J. hieronymi* compared with that of *J. excisa*. *Jatropha hieronymi* seeds are round, light brown coloured and without mottlings, whereas *J. excisa* seeds are oblong, light brown to black coloured with a mottled pattern. Both seeds have a very hard coat. Elaiosomes of both species are white, soft and firmly attached to the micropylar end of the seed. The elaiosome mass of *J. hieronymi* is 40-fold higher than that of *J. excisa* and the elaiosome/seed ratio is much greater for *J. hieronymi* than that for *J. excisa* (Table 1).

Fruits of *J. hieronymi* are subglobose, whereas *J. excisa* fruits are elliptic and considerably smaller than those of *J. hieronymi* (Fig. 1C,D; Table 1). The fruit wall of both *J. excisa* and *J. hieronymi* has an external epidermis (exocarp), an intermediate photosynthetic parenchyma (mesocarp) and an inner mechanical layer (endocarp). The mechanical layer consists of two sublayers of fibre sclereids, enclosing a palisade-like sublayer of sclereids with a big lumen. The sclereids of both sublayers are dead cells and have lignified walls. Both types of sublayers are oriented at right angles to each other, with the outer sublayers parallel to the wall surface and the inner sublayer perpendicular to it (Fig. 2). The entire mechanical layer of *J. hieronymi* fruits after dehiscence is more than 4-times thicker

Table 1. Seed and fruit measurements of *Jatropha hieronymi* and *J. excisa*

	<i>J. hieronymi</i>	<i>J. excisa</i>
Seed mass (mg) [†]	511.7 ± 97.8	60.7 ± 11.8
Percent water	1.6	1.94
Elaiosome mass (mg) [†]	84.55 ± 12	2.32 ± 0.53
Elaiosome/seed ratio	0.198	0.039
Seed length (mm) [‡]	14.14 ± 0.7	7.73 ± 0.66
Seed width (mm) [‡]	10.07 ± 0.3	4.74 ± 0.2
Fruit length (mm) [‡]	21.8 ± 1.4	10.8 ± 0.5
Fruit width (mm) [‡]	23.4 ± 1.7	9.14 ± 0.5
Mechanical wall thickness (mm) [‡]	1.9 ± 0.04	0.44 ± 0.01

Values are means ± SD. [†]*n* = 80 seeds. [‡]*n* = 30 fruits.

than that of *J. excisa* (Table 1), and the outer sublayer of *J. hieronymi* endocarp is composed of 16 ± 2 rows of fibre sclereids, whereas that of *J. excisa* is composed of 8 ± 2 rows.

Fatty acid composition of the elaiosomes

The elaiosome oil content was slightly higher for *J. hieronymi* than for *J. excisa* (Table 2). The fatty acids composition of the elaiosomes consisted mainly of unsaturated to polyunsaturated chains with 16–18 C units and were similar to the fatty acid composition of elaiosomes of many other myrmecochorous species (Hughes *et al.* 1994a; Pizo & Oliveira 2001). In the elaiosomes we found oleic, palmitic, stearic and linoleic fatty acids, in decreasing order of abundance. *Jatropa hieronymi* had a significantly higher percentage of oleic acid than *J. excisa*, as well as lower linoleic and stearic acid. Palmitic acid percentage was slightly higher for *J. hieronymi* (Table 2).

Explosive seed dispersal

The larger and heavier seeds of *J. hieronymi* were thrown significantly further distances than were *J. excisa* seeds.

Jatropa hieronymi dispersal distances ranged from 0.52 to 17.62 m (mean \pm SD = 6.91 ± 0.42 m, median = 6.65 m, $n = 226$) with a modal peak between 6 and 7 m and a long tail that exceeded 17 m. *Jatropa excisa* dispersal distances had a modal peak between 0.8 and 1 m (mean \pm SD = 1.97 ± 1.37 m, median = 1.68 m, $n = 370$), and the range that the seeds were thrown was from 0.1 to 7.65 m (Fig. 3A,B). The distance of explosive dispersal was statistically different between species based on the Mann–Whitney *U*-test ($U = 421.5$, $Z = 7.49$, $P < 0.0001$).

Differences in seed weight within species did not significantly influence dispersal distances ($r = 0.0064$, $t_{73} = 0.055$, $P = 0.95$ for *J. hieronymi*; and $r = 0.0096$, $t_{50} = 0.068$, $P = 0.94$ for *J. excisa*).

Seed removal by ants

Ants removed a significantly greater number of seeds of *J. excisa* (200 from 240 seeds) from the cardboard plates than of *J. hieronymi* (76 from 240 seeds, $\chi^2 = 128.98$, d.f. = 1, $P < 0.0001$). Within the first hour of the experiment, ants found the seeds of both species and were attracted to them. Ants gnawed the elaiosome or dragged the seeds to the nest entrance, holding the elaiosome in their mandibles (Fig. 1E,F). We did not

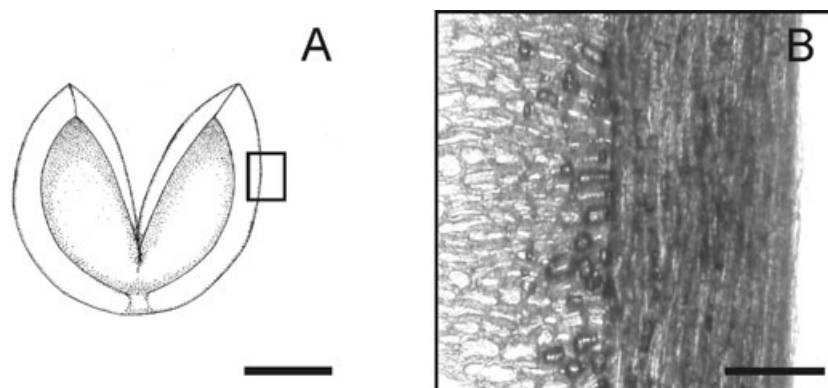


Fig. 2. Fruit wall structure of *Jatropa hieronymi*. (A) A single carpel after dehiscence, only the remaining lignified mechanical layer (endocarp) is shown. Scale bar: 6 mm. (B) Light microscopy detail of the mechanical layer, showing the sclereids of the outer and inner sub-layers, and the right angle orientation respect each other. Scale bar: 150 μ m.

Table 2. Oil content and fatty acid profile of elaiosomes of *Jatropa hieronymi* and *J. excisa*

Species	Total oil	Palmitic 16:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2
<i>J. excisa</i>	32.35 ± 1.56	23.61 ± 0.04	18.94 ± 1.02	37.71 ± 0.06	13.74 ± 0.07
<i>J. hieronymi</i>	35.13 ± 0.02	25.11 ± 0.09	6.88 ± 0.08	55.06 ± 0.08	3.68 ± 0.08
<i>F</i> -value	9.68	695.87	416.82	90306.75	26868.21
<i>P</i>	<0.05	<0.005	<0.005	<0.005	<0.005

Elaiosome oil content is expressed as percentage on dry weight basis. Fatty-acid profile is expressed as percentages of total seed oil content. Data are mean \pm SD of triplicate determinations. Comparison of means between the two species was analysed using one-way ANOVA.

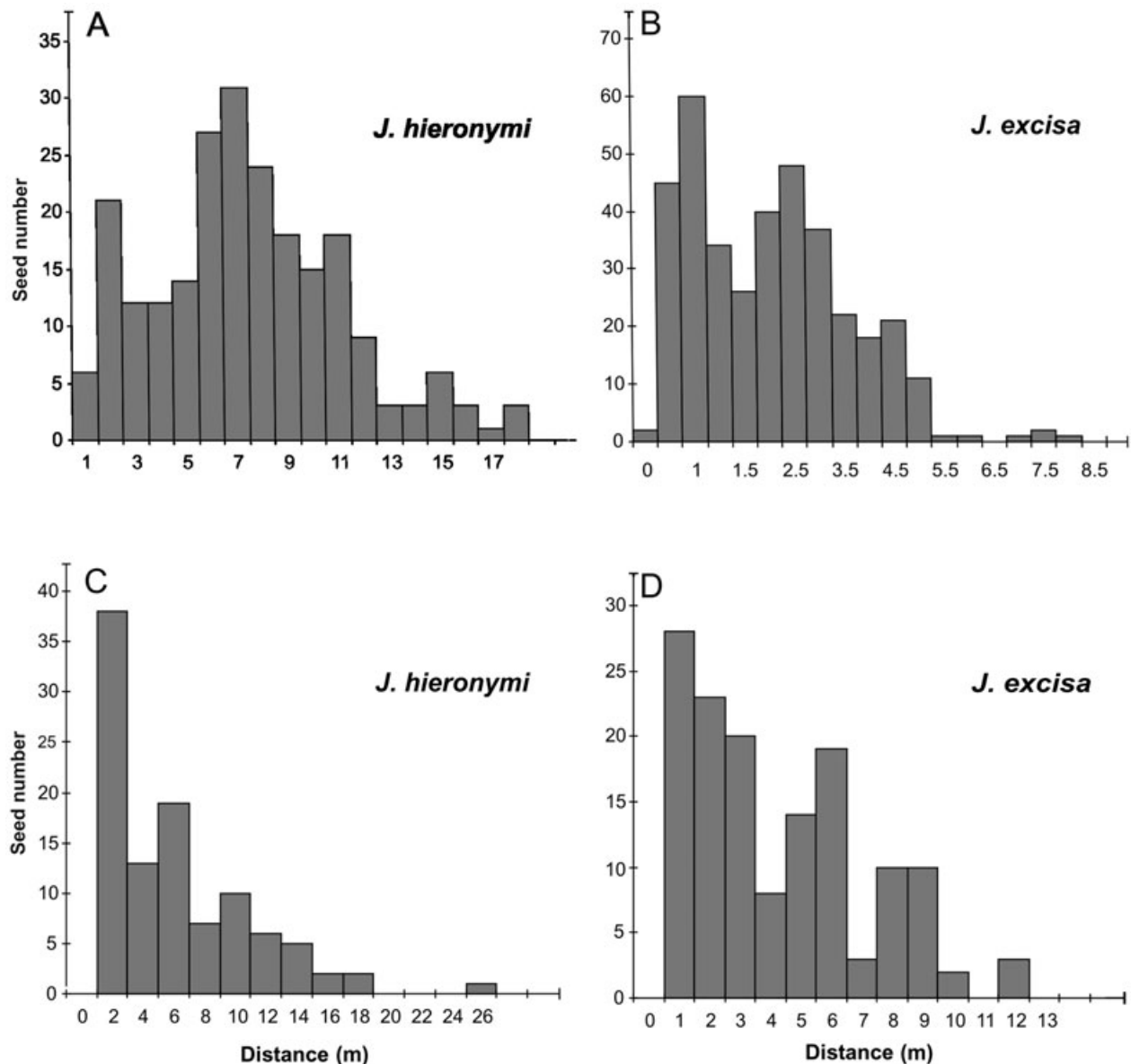


Fig. 3. Frequency histogram of dispersal distances of seeds of *Jatropha hieronymi* and *J. excisa*. (A,B) Dispersal distances by explosive dehiscence under experimental conditions, $n = 226$ for *J. hieronymi* seeds and $n = 370$ for *J. excisa* seeds. (C,D) Dispersal distances by ants in mixed natural populations of the plant species. Distances were obtained from direct observations of 140 seed transports for *J. hieronymi* and 103 seed transports for *J. excisa*. Values represent the upper limits of the class on the x-axis.

observe ants detaching the elaiosome from seeds in order to take only the appendage to their nest, but small ant species gnawed the elaiosome of *J. hieronymi* and *J. excisa* without transporting the seeds. Only large bodied ants were observed carrying the seeds of *J. hieronymi*.

Analysis of the number of seeds removed from the seed plate pairs with and without elaiosomes showed that seeds with their elaiosome attached were preferentially removed by ants. The number of *J. excisa* elaiosome-bearing seeds removed at the end of the experiment was almost threefold greater than that of seeds without elaiosome (201 vs. 69 from 240 seeds, $\chi^2 = 145.28$, d.f. = 1, $P < 0.0001$); whereas removal of

J. hieronymi elaiosome-bearing seeds was almost five-fold greater than that of seeds without elaiosome (67 vs. 14 from 240 seeds; $\chi^2 = 40.16$, d.f. = 1, $P < 0.0001$, Fig. 4). Both the presence/absence of elaiosome and the plant species significantly affected the number of seeds removed, but no significant interaction between these factors was found (Table 3).

Seed dispersal distance by ants

A total of 103 seed transports were recorded for *J. hieronymi* and 140 for *J. excisa*. Mean transport dis-

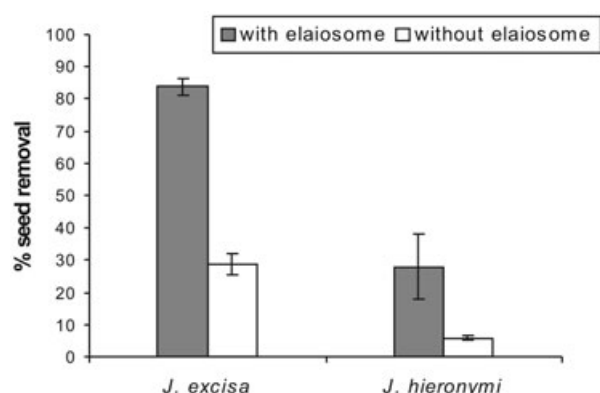


Fig. 4. Removal by ants of *Jatropha excisa* and *J. hieronymi* seeds with and without elaiosomes. Mean percentage of seeds removed in the field by ants from seed plate pairs with and without elaiosomes after 12 h of experiment. Eighty seeds per treatment of each species were used in each trial ($n = 3$). Bars indicate standard deviation.

Table 3. Two-way ANOVA for the effects of plant species and elaiosome presence/absence on the number of seeds removed by ants

Effects	d.f.	Mean square	F	P
Plant species	1	21.512	168.295	<0.0001
Elaiosome presence/absence	1	20.033	156.722	<0.0001
Species \times elaiosome	1	0.05	0.391	0.534
Error	64	0.127		

The dependent variable (number of seeds removed) was log ($x + 1$) transformed prior to the analysis.

tance for *J. hieronymi* was 5.22 ± 4.80 m (mean \pm SD, median = 4.1 m), and the maximum dispersal distance was 25.6 m. *Jatropha excisa* seeds were transported a mean distance of 3.70 ± 2.9 m (median = 2.9 m) and the maximum dispersal distance was 12 m (Fig. 3C,D). Ant dispersal curves for both species had a narrow peak at short distances and a long tail, where the number of dispersed seeds decreased with increasing dispersal distances. Mean dispersal distance by ants was significantly lower than explosive dispersal distance for *J. hieronymi* (Mann–Whitney U -test, $U = 8007.5$, $Z = -4.45$, $P < 0.0001$). In contrast, *J. excisa* mean transport by ants was further than that achieved by explosive dispersal ($U = 17599$, $Z = -5.58$, $P < 0.0001$).

Removal by vertebrates

Seeds were not removed from the plates after four days of the experiment. Foxes have been suggested to be one of the most important seed dispersers in this region (O. Varela, pers. comm., 2008), but we have never found

Jatropha seeds in foxes feces dropped around each experimental area (unpublished data). These observations could suggest that ants are the principal consumers/dispersers of these seeds in the study region.

DISCUSSION

Our results confirm that *J. excisa* and *J. hieronymi* exhibit two sequential seed dispersal mechanism, autochory through explosive dehiscence of the fruit and myrmecochory through dispersal by ants.

Autochory

These results illustrate the potential of primary explosive dispersal of *J. excisa* and *J. hieronymi* seeds under controlled conditions. Maximum dispersal distances of both species were considerably greater than the typical distances of less than 5 m found in other species with explosive dispersal (see review by Hughes *et al.* 1994b). In certain cases, distances up to 9 m have been reported, but maximum distances of more than 20 m have only been recorded for non-myrmecochorous trees more than 10 m height (Swaine & Beer 1977). The observed mean and maximum dispersal distances of *J. hieronymi* under controlled conditions seem to be one of the greatest recorded in the literature for a diplochorous species.

According to Garrison *et al.* (2000) differences in seed mass should cause a difference in dispersal distances, where the heaviest seeds might be expected to fall closer to the parent plant. Nevertheless, no clear relationship between seed mass and explosive dispersal distance has been reported (Schmitt *et al.* 1985; Lisci & Pacini 1997; Berg 2000; Narbona *et al.* 2005). In this study, we did not find a relationship between seed mass and dispersal distances at intraspecific levels but a strong positive correlation at interspecific levels, in that heavier *J. hieronymi* seeds were thrown greater distances than lighter *J. excisa* seeds. Likewise, this positive correlation between seed mass and dispersal distance is surprising, because (i) a heavier seed should fall to the ground sooner than a lighter seed (Harper *et al.* 1970) and (ii) seeds explosively dispersed usually weigh less than 100 mg (Hughes *et al.* 1994b), compared with >500 mg for *J. hieronymi* seeds.

This paradox can be explained by the fact that in *J. hieronymi* larger seeds are produced by larger fruits, with thicker lignified mechanical layers than *J. excisa* fruit walls. The mechanical layer of the fruit wall is responsible for the explosive dehiscence of the seeds (Berg 1990; Lisci & Pacini 1997). The different sclereids sublayers of the mechanical layer are oriented at right angles with respect to each other. As the fruit ripens, the exocarp and mesocarp dry out and the inner mechanical layer dehydrates. The sublayers of

the endocarp pull against each other giving rise to tension forces that cause the opening of the capsules along the dehiscence lines, and finally the walls curl, ejecting the seeds. The thicker mechanical layer of the *J. hieronymi* fruit wall develops a proportionately stronger explosive force at ripening that is then transferred to the ejected seed. Additionally, the rounded shape of the *J. hieronymi* seeds, almost a perfect sphere when seen without the elaiosome, provides a minimal air resistance during the projection of the seed. Beer & Swain (1977) have proposed that the initial velocity of the projected seed and its air resistance have a greater influence on dispersal distances than other factors, like the height of release or the angle to the horizontal at which the seed is projected. Within Fabaceae species with explosive dispersal, Schmitt *et al.* (1985) have found that legume size plays a decisive role in the projection power, and hence the distance travelled by explosively dispersed seeds. To the best of our knowledge, there are no other data on the relationship between fruit size, seed mass and dispersal distances for explosively dispersed seeds of other plant families. Most studies have focused on the seed rather than on the fruit characteristics.

In diplochorous plants, a trade-off between the morphological requirements for long-distance explosive dispersal and the use of secondary dispersal agents has been suggested such that plants may maximize either the primary or the secondary seed dispersal event (Beattie & Lyons 1975; Stamp & Lucas 1983; Ohkawara & Higashi 1994). It has been postulated that the presence of the elaiosome alters the seed aerodynamics and reduces the explosive dispersal distance (Beattie & Lyons 1975). In two species of *Viola*, the species with greater explosive dispersal distance has a markedly smaller elaiosome (Ohkawara & Higashi 1994), and in two species of *Euphorbia*, the interspecific differences in dispersal distances was due to the loss or conservation of the caruncle during the dehiscence (Narbona *et al.* 2005). In contrast, *J. hieronymi* and *J. excisa* always conserve their elaiosome after the explosion of the capsule, and *J. hieronymi* achieved the greater explosive dispersal distance despite having a relatively big elaiosome. Thus, *J. hieronymi* is not favouring one dispersal mode over the other, and the effectiveness of both primary and secondary dispersal is not altered.

In natural populations of *J. excisa* and *J. hieronymi*, the dispersal distances by explosive dehiscence could differ from results obtained here: surrounding vegetation and wind speed as well as soil obstacles probably affect the dispersal curve under natural conditions. At the very least, these species have the potential to disperse their seeds far away from the competition zones of both mother and siblings, and this is probably the adaptive value of the highly efficient explosive dispersal in these species. Currently, attempts to model

dispersal are focusing increasingly on tails of distance distributions, as the small proportion of seeds that travel long distances play a key role in the colonization of new areas (Malo 2004). In arid environments, a long dispersal distance should increase the probability that at least some seeds will end up at suitable sites.

Myrmecochory

Jatropha excisa and *J. hieronymi* seeds with elaiosome were more attractive to ants than seeds without elaiosome, a pattern demonstrated before by other authors for myrmecochorous seeds (O'Dowd & Hay 1980; Davidson & Morton 1981; Byrne & Levey 1993). Ants were rapidly attracted to the elaiosome, but not to the seed body itself. When dragging the seeds to the nest, the elaiosome acted as a handle for the ants facilitating the seed transport, this handle function has already been highlighted and could be an important evolutionary force in this type of ant-plant mutualism (O'Dowd & Hay 1980; Horvitz 1981; Gómez *et al.* 2005). Numerous studies have noted that seeds adapted for ant dispersal tend to have hard coats (Berg 1975; Beattie 1985; Higashi *et al.* 1989; Rodgers 1998), as have both species in this study. Westoby *et al.* (1982) suggested this may act to protect seeds from predation, and thus increase the chance that the seeds are effectively dispersed. Another possible defence mechanism to avoid ant seed predation is the use of chemical defences. Toxic compounds have been found in seeds of *Datura discolor* (O'Dowd & Hay 1980; Bell 1984) and *Ricinus communis* (Bell 1984), both of which possess elaiosomes. Seeds of *J. hieronymi* and *J. excisa* have been reported as toxic, and native people use them for medicinal purposes such as purging (Ratera & Ratera 2007). These defences may also act against vertebrate predators (rodents, birds and foxes in this region). Exclusion experiments in this study showed that ants are the only agents removing these seeds at least in the study areas.

The number of seeds removed by ants was higher for *J. excisa* than for *J. hieronymi*. Between species, the quantity of seeds removed by ants was negatively influenced by seed size as the larger seeds of *J. hieronymi* were removed to a lesser extent than the smaller seeds of *J. excisa*. Other authors have found that small diaspores were removed in greater numbers and transported to greater distances by a more diverse ant assemblage than large diaspores (Gorb & Gorb 1995; Pizo & Oliveira 2001). Also, it has been shown that dispersal of large seeds depends on the presence of ant communities with large body size, which are physically able to lift and remove the seed from the ground (Pizo & Oliveira 2001; Ness *et al.* 2004).

Selective forces on this ant-plant mutualism type should favour increasing elaiosome size or elaiosome/

seed ratio. It has been proposed that ants might not select seeds based on elaiosome mass per se, but rather on some measure of the quality of the elaiosomes such as lipid content and especially oleic acid content, as oleic acid is proposed to be a stimulant for seed-carrying behaviour in some ant species (Lanza *et al.* 1992; Hughes *et al.* 1994; Edwards *et al.* 2006). In the present study, despite the huge differences in elaiosome size between *J. hieronymi* and *J. excisa* (19.8% vs. 3.9% of seed mass, respectively), the percentage of seed removal was greater for *J. excisa*. Moreover, although the analysis of the lipid content of the elaiosomes revealed significant differences in total oil content and fatty acid composition between the two species with significantly higher oleic acid content in *J. hieronymi* elaiosomes, these differences were not reflected in the quantity of seeds removed by ants. Thus, the efficiency of myrmecochorous dispersal for these two *Jatropha* species may depend more on the size-matching effect between seeds and ant species than on the elaiosome properties.

Jatropha hieronymi seed dispersal distance by ants was greater than that of *J. excisa*, and was positively correlated with seed size between the two species. Ant species with bigger workers and a relative lower nest entrance density may explain the long tail of the dispersal curve (Gómez & Espadaler 1998a). Larger ants tend to forage further, and transport seeds further than small ants. Also the density of nests of large ants is much lower than that of small ants (Pudlo *et al.* 1980; Hughes & Westoby 1992). As indicated by Giladi (2006), the overall fitness gain for plants resulting from myrmecochory significantly depends on the identity and behaviour of the seed disperser.

The observed ant dispersal distances for *J. hieronymi* (mean 5.22 m and maximum 25.6 m) and for *J. excisa* seeds (mean 3.70 m and maximum 12 m) are large compared with the typically short distances (in average less than 1 m) reported in the world literature (Gómez & Espadaler 1998b). The long dispersal distances by ants in this study are in the range of those reported for plants from the Sonora Desert (O'Dowd & Hay 1980), Australian sclerophyllous arid zones (Andersen 1988) and South African Fynbos (Slingsby & Bond 1985). Dispersal curves with long tails are most advantageous for plant species with rare safe sites (Green 1983), and this should be also the case in the Monte Desert where the climate and infertile soils suggest similar ecologic conditions to the regions mentioned above. Finally, the ant dispersal distances in this study support the hypothesis that in the Southern Hemisphere, and in particular in arid environments, ant dispersal distances tend to be greater compared with other areas of the world (Whitney 2002). Further studies should be carried out to determine the key advantages of diplochory at both local and regional ecological scale.

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