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# INSECT POLLINATORS OF *JASMINOCEREUS THOUARSII*, AN ENDEMIC CACTUS OF THE GALAPAGOS ISLANDS

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## SUMMARY

*Jasminocereus* is a columnar cactus endemic to the Galapagos Islands, and is distributed mainly in the lowland arid zones. Its only species, *J. thouarsii*, has several varieties on different islands. Observations of the variety *J. thouarsii* var. *delicatus* on Santa Cruz Island suggested limited recruitment. We therefore studied its floral biology, pollination requirements and seed germination to determine whether recruitment might be limited by seed production or seed quality. Flowers opened in the early morning, from 5h00 to 10h00. No seed was produced by flowers isolated in pollination bags. Pollination was allogamous, mostly brought about by the endemic *Xylocopa darwini* (Hymenoptera: Apidae), followed by the introduced *Acrosticta apicalis* (Diptera) and endemic *Camponotus planus* (Hymenoptera: Formicidae). Seeds germinated well, with the highest germination rate obtained from seeds that were soaked before planting.

## RESUMEN

**Insectos polinizadores de *Jasminocereus thouarsii*, un cactus endémico de las Islas Galápagos.** *Jasminocereus* es un cactus columnar endémico de las Islas Galápagos que se encuentra principalmente en las zonas áridas de baja altitud. Su única especie, *J. thouarsii*, posee algunas variedades en diferentes islas. Observaciones de *J. thouarsii* var. *delicatus* en la Isla Santa Cruz, sugieren que existe una renovación limitada de la población. Por lo tanto, se estudió su biología floral, requerimientos para su polinización y germinación de las semillas para determinar si el reclutamiento podría estar limitado por la producción de semillas o por la calidad de las mismas. Las flores se abrieron por la mañana, entre las 5h00 y las 10h00. Las flores aisladas en bolsas antipolinizadores no produjeron semillas, ya que la polinización es de tipo alógamo, mayormente llevada a cabo por *Xylocopa darwinii* (Hymenoptera: Apidae; endémico), seguida por *Acrosticta apicalis* (Diptera; introducida) y *Camponotus planus* (Hymenoptera, Formicidae; endémica). Las semillas germinaron bien, habiéndose obtenido el mayor porcentaje de germinación a partir de semillas que fueron puestas en remojo antes de ser plantadas.

## INTRODUCTION

*Jasminocereus thouarsii* (Weber) Backbg. (Cactaceae) is the only species of the Galapagos endemic genus *Jasminocereus*. It is a columnar cactus distributed in the arid coastal areas of several islands. It is listed as Near-Threatened (León-Yánez *et al*. 2010), and preliminary studies have shown that natural regeneration is infrequent (P.J. unpubl.).

Three varieties of *Jasminocereus thouarsii* have been recognized (Wiggins and Porter 1971): var. *sclerocarpus* (K.

Schum.) Anderson & Walkington on Fernandina and Isabela; var. *thouarsii* (Weber) Backbg. on Floreana and San Cristobal; var. *delicatus* (Dawson) Anderson & Walkington on Santa Cruz, Santiago and other smaller islands such as Bartolomé (McMullen 1999). The variety studied here, *J. t.* var. *delicatus*, is a branched columnar cactus up to 7 m tall. It flowers between December and June. Flowers are 6–8 cm in diameter, yellowish with numerous stamens 2 cm long and a stigma of 1–1.5 cm. The reddish purple fruits are 1.5–4.5 cm long and 3.5–4.2 cm across (Wiggins & Porter 1971).

*J. thoursii* is listed as Near Threatened (León-Yánez *et al.* 2010), and preliminary studies have shown that natural regeneration is infrequent (P.J. unpubl.). The species has no asexual reproduction and there is no clear indication of the causes of limited regeneration. Self incompatibility and low seed production have been observed in other cactus species (*e.g.* Mandujano *et al.* 1998, Piña *et al.* 2007), which led us to investigate the reproductive biology of *J. thouarsii*.

Numerous species of columnar cacti are pollinated by bats (Valiente-Banuet *et al.* 2002) but Galapagos bats are exclusively insectivorous (McCracken *et al.* 1997). Previous studies have shown that the endemic carpenter bee *Xylocopa darwini* Cockerell (Hymenoptera: Apidae) is the main pollinator for many Galapagos flowering plants (McMullen 1987). *Opuntia* flowers are visited by finches Geospizinae (B.R. Grant & Grant 1981), which eat pollen (Grant 1996) but no study mentions visits to *Jasminocereus*. Jackson (1993) predicted nocturnal insects (especially moths) as the pollinators of *Jasminocereus*, but the characteristics of the flowers suggest that pollination by the carpenter bee may be more likely.

This paper describes aspects of the reproductive biology of *J. thouarsii*, including the flowering phenology, insect visitors and pollen grain structure, and reports experiments to determine whether the species requires a pollen vector, optimal conditions for seed germination, and the growth rate of seedlings.

## METHODS

The study was performed in the dry coastal zone of Santa Cruz Island, Galapagos, Ecuador, in the area surrounding the Charles Darwin Research Station (*c.* 0°44´S, 90°18´W). The climate is tropical semi-arid with an average annual rainfall of 620 mm (CDRS weather station for the period 1982–2002) but with extreme variability due to the El Niño Southern Oscillation that causes most years to have either much higher or lower precipitation (*e.g.* 2768 mm in 1983, 63 mm in 1985). Rainfall in coastal areas is highly seasonal with a cool dry season from July to September (average daily temperatures around 21°C) and a hot wet season from December to April (26–27°C). The study was carried out in 2001 and 2002 during the hot wet season when flowering takes place. The first year of study (2001) was dry, with 293 mm rainfall, the second year (2002) near average (577 mm). Being located close to the equator, the sun rises within about 30 minutes of 6h00 local time throughout the year

Ten *Jasminocereus* adults were selected in the study area and tagged. Daily flowering phenology of 25 flowers on these plants was observed during two weeks in April in 2001 and 2002, in the middle of the flowering season. For the two plants with the most flowers, eight flowers per plant were selected for study of pollinator visits. Flowers were open between 6h00 and 11h00 only. Three observers identified and counted all visitors to the 16 flowers during the first 20 minutes of each hour from 6h00 to 11h00, for 14 days each year in May 2001 and 2002. Additional night observations were carried out between 18h00 and 22h00 for four nights in 2001. Following these observations, all 16 flowers studied were harvested for collection and description of pollen.

Pollen grains were acetolysed following the protocol of Erdtman (1960) and Kearns & Inouye (1993), and mounted in glycerine jelly for light microscopy. Measurements were made with the light microscope on 25 pollen grains. For scanning electron microscopy, the acetolysed pollen was mounted on cover slips previously attached to aluminium stubs with silver paint, coated with evaporated gold by ion sputtering and examined with a JEOL JSM 840 microscope. The terminology used for pollen descriptions follows Punt *et al.* (1994).

On each of five of the ten plants, two flower buds were tagged and enclosed in Hubo "golden magic-mark" pollination bags to test for self pollination. On each of the other five plants, two unenclosed buds were tagged as open pollination controls. Fruits produced were harvested when ripe and seeds were counted.

From each of the five control plants, five additional mature fruits per plant (total 25 fruits) were harvested in June 2001, to obtain 1200 seeds for germination trials. Three replicates of 100 seeds were submitted to each of four treatments: in T1 and T2 seeds were submerged for 24 h in water at a constant temperature of 25 and 60°C respectively and then laid on moist filter paper in petri dishes; in T3 and T4 seeds without prior soaking were laid in petri dishes on humid soil and on moist filter paper respectively. Petri dishes were placed next to a window inside a laboratory with no additional light or heating for four months. When seedlings reached 5 mm in height, they were transplanted to soil and measured monthly until April 2002. Seedlings were watered regularly.

## RESULTS

### Flowering phenology

During our study, all flowers opened in the morning after 5h30 and closed by 10h00. None opened at night.

### Insect visitors

In a total of 800 recorded insect visits during both years, the only three insect species recorded were *Xylocopa* *darwini* (54 % of visits), the introduced fly *Acrosticta apicalis* Williston (Diptera: Ulidiidae) (34 %) and the endemic ant *Camponotus planus* Smith (Formicidae) (12 %). For all species, a peak was observed between 7h00 and 7h20. Less than 3 % of the visits were between 10h00 and 10h20. No other animal was observed visiting the flowers during the study. No insect was observed visiting the closed flowers during the night. The cumulative number of visits during the morning hours was significantly larger in 2002 than 2001 for the three insect species; the peak was the same each year with an overall average of about two visits per 20-minute observation period (Fig. 1). The pattern of variation in time was similar in both years as indicated by the absence of a year–time interaction in a three way ANOVA (Table 1).

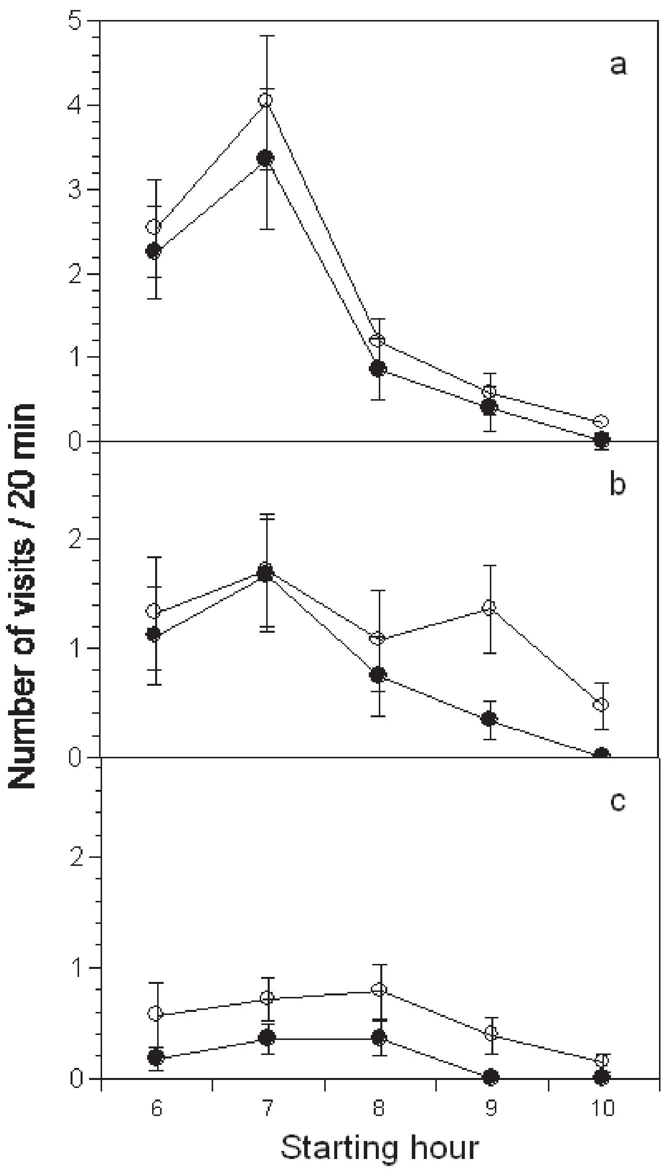


**Figure 1.** Visits to *Jasminocereus thouarsii* flowers in May 2001 (closed circles) and May 2002 (open circles) by three insect species: a. *Xylocopa darwini*, b. *Acrosticta apicalis*, c. *Camponotus planus.* Data are mean ± SE (n = 28: 14 days x 2 plants).

**Table 1.** Results of a three way ANOVA for the visits of three insect species to flowers of *J. thouarsii* in 2001 and 2002, and between 6h00 and 10h00.

| Effect | d.f. | F | *P* |
|---|---|---|---|
| Species | 2 | 25,4 | 0,0000 |
| Year | 1 | 7,11 | 0,0078 |
| Time | 4 | 22,0 | 0,0000 |
| Species.Year | 2 | 0,03 | 0,9662 |
| Species.Time | 8 | 7,10 | 0,0000 |
| Year.Time | 4 | 0,11 | 0,9792 |

**Pollen description**

Pollen grain morphology was studied in 10 selected plants but only one sample was acetolyzed for scanning electron microphotography. This plant and the acetolyzed pollen sample are deposited in the Charles Darwin Research Station Herbarium as sample CDS 11771. Fresh pollen grains tended to agglomerate due to the presence of fat compounds. Pollen grains (Fig. 2) were trizonocolpate, sometimes trizonocolporoidate, isopolar, radiosymmetric, circular and slightly 3-lobed with convex mesocolpia in polar view and circular to slightly elliptical in equatorial view, from suboblate to prolate-spheroidal. Polar/ Equatorial (P/E) axis ratio was 0.85–1.02 (mean 0.96, n = 25 grains), with P = 36–44 (mean 39.6) µm and E = 39–44 (41.4) µm. The apertures were terminal, long and narrow colpi with colpal membrane scabrate to granular, a diffuse pore appearing in the equatorial zone, generally only well appreciable with the light microscope. Exine was 2–3 µm thick, with the sexine being c. three times thicker than the nexine. Infratectum was columellated, tectum perforate, the perforations circular, surrounded by a thick

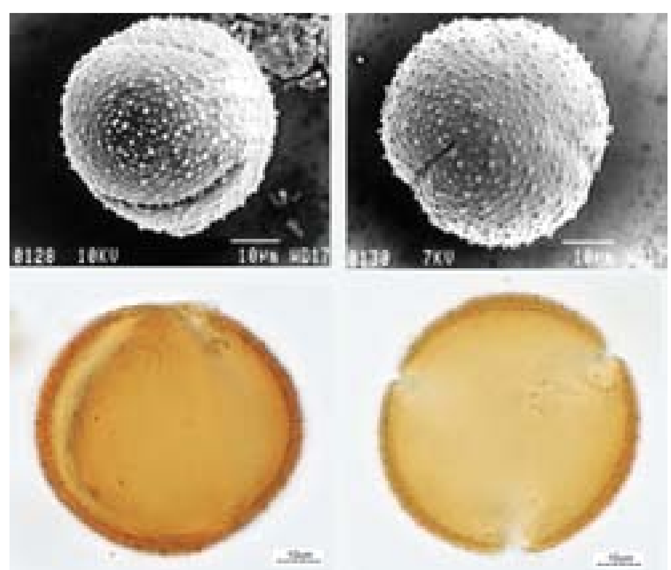

**Figure 2.** Top, scanning electron micrographs of *Jasminocereus* pollen (left, equatorial view; right, polar view); bottom, light microscope images (left, equatorial view; right, polar view).

border and regularly disposed all around the surface of the pollen grain. As supratectal elements, we could distinguish dispersal, conical and bulbous microechinae with a wider base and striated surface about 2 μm in diameter and 1 μm high.

**Pollination mechanism**

None of the flowers enclosed in pollination bags produced fruit. The control flowers all produced fruits that reached about 2 cm long after 15 days. They were ripe and harvested after 22 days. Mean seed production was 325 seeds per fruit (SE 15.3, n = 10).

**Seed germination**

Seed germination was high in all treatments apart from T3 (unsoaked, on soil). Differences among treatments were significant (ANOVA $F_{3,8} = 27.9$, $P < 0.0001$) but the post-hoc Tukey HSD test showed that only the treatment in soil differed from the others (mean 7.3 %, SE 4.6, n = 3 in soil vs 54.0 %, SE 3.5, n = 9 for the other three treatments, Table 2). Germination started after four days following treatments T1 and T2, after 20 days in T3 and eight days in T4.

**Seedling growth**

During the growth measurements between June 2001 and April 2002, mortality was high for all treatments, decreasing after month 10. The height (ground to tip) of 11-month old seedlings ranged between 9 and 25 mm. Differences among treatments were significant, with maximum growth for T2 (ANOVA, $F_{3,106} = 4.72$, $P = 0.004$, Table 2).

## DISCUSSION

This study presents baseline information that will help to determine factors that may be significant in the recruitment of this endemic species.

Although animals are known to be frequent pollen vectors for columnar cacti (Fleming *et al.* 2001, Clark-Tapia & Molina-Freaner 2004, Ibarra-Cerdeña *et al.* 2005), in Galapagos, where all the bat species are insectivorous, *Jasminocereus* appears adapted to insect pollination. The timing of flower availability (morning opening, closing by 10h30) and the pollen characteristics (sticky, nutritious and large) are indicative of entomophilous pollination. Contrary to what Anderson (2001) mentions, our observations show flowers were closed at night, with no nocturnal pollinators.

Numerous flowering plants in Galapagos rely on one generalist pollinator, the endemic carpenter bee *Xylocopa darwini*, that is known to visit some 60 plant species (Linsley *et al.* 1966, McMullen 1987, 1993). Our observations confirm the importance of this pollinator and represent the first records of insect pollinators for *Jasminocereus* in the Galapagos Islands.

Comparing fruit and seed set of open-pollinated flowers versus flowers that were isolated from pollinators, we conclude that *Jasminocereus* is not capable of autonomous self-pollination. Of 52 Galapagos plant species studied by McMullen (1987), 40 were self-compatible. *Jasminocereus* may be self-compatible (autogamous), but requires a pollen vector.

In the case of *Jasminocereus*, the need for a vector could well be a cause for the limited fruit production of isolated plants. For plants receiving adequate pollinator visits to produce fruits however, viable seed production per fruit appears to be sufficient for this not to be a limiting factor in the regeneration of the species. However, the low germination success for seeds planted directly into soil, a drier medium than filter paper in petri dishes, suggests that germination in the field may be rare, perhaps associated with high rainfall events that occur with El Niño.

Another important factor to take into account, and not measured here, is loss of flowers and fruit to herbivores. Finches and mockingbirds *Nesomimus* spp. eat and destroy flowers and flower parts of some cacti (P.R. Grant & Grant 1979, B.R. Grant & Grant 1981, Millington & Grant 1983, Grant 1996) as do lava lizards *Microlophus* spp. (C. Buddenhagen pers. comm.); this may limit seed production in some cases.

Information on the reproductive biology of endemic species is a critical step to understanding factors that may limit their populations. The life history strategies of long-lived species such as *Jasminocereus* may be especially difficult to understand, as they may be highly adapted to irregular El Niño Southern Oscillation events. Thus long term study is essential for gathering the information necessary to set appropriate conservation priorities.

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**Table 2.** *Jasminocereus thouarsii* germination trial results for four treatments (see Methods). Data are mean ± SE (n).

| | T1 | T2 | T3 | T4 |
|---|---|---|---|---|
| Final germination % | 52.0 ± 10.2 (3) | 47.3 ± 8.1 (3) | 7.3 ± 3.0 (3) | 66.7 ± 8.6 (3) |
| Seedling mortality % | 43 | 48 | 30 | 47 |
| Final growth in length after 11 months (mm) | 13.3 ± 1.8 (36) | 16.4 ± 4.9 (29) | 14.2 ± 0.4 (7) | 14.4 ± 3.1 (38) |

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