Parthenocarpy and Seed Predation by Insects in Bursera morelensis

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INTRODUCTION

Seed development is one of the key processes in angiosperm reproduction (Yadegari and Drews, 2004; Berger et al., 2006). The abortion of flowers, fruits and seeds is a physiological process in which plants can fit progeny levels according to resource availability avoiding predator-damaged seeds or genetically deficient ones (Janzen, 1971, 1977; Sorensen, 1982; Stephenson and Bertin, 1983; Evenari, 1984).

All species of the genus Bursera (Burseraceae) have seeds covered by a pseudoaril that has a colour contrasting with surrounding vegetation (Rzedowsky et al., 2004, 2005). These seeds possess all the features to be bird dispersed (Howe and Westley, 1988). Several studies have stressed the importance of seed dispersion of Bursera by birds (Scott and Martin, 1984; Trainer and Hill, 1984; Bates, 1992; Greenberg et al., 1993; 1995; Poulin et al., 1994; Grant and Grant, 1996; Ortiz-Pulido et al., 1999; Ortiz-Pulido and Rico-Gray, 2000; Graham, 2002; Stevenson et al., 2005) and other vertebrates (Clark and Clark, 1981; Evans, 1989; Stevenson, 2000; Stevenson et al., 2000). The genus has more than 100 species of trees and shrubs inhabiting tropical dry forests (Espinosa et al., 2006). Development of fruits and seeds in the genus are almost unknown except for Srivastava (1968) and Cortes (1998). Cortes (1998) described the production of apomictic seeds in Bursera medranoana, while Srivastava (1968) and Cortes (1998) reported that the development of ovules with respect to the ovary in B. delpechiana and B. medranoana suffered a delay giving the impression of a fruit without seed. The presence of parthenocarpic fruits has been reported for B. fagaroides and B. morelensis (Verdú and García-Fayos, 1998).

The production of parthenocarpic fruits has been regarded as a defensive strategy to lower predation probabilities of viable seeds (Zangerl et al., 1991; Traveset, 1993a, b; Ziv and Bronstein, 1996; Fuentes and Schupp, 1998; Verdú and García-Fayos, 1998, 2001). Insects lay eggs into fruits when ovules are still immature without knowing if the fruit will bear seeds. When fruits become mature only those larvae in fruits with seeds can survive (Scurlock et al., 1982; Coetzee and Gillooly, 1987; Jordano, 1989, 1990; Niwa and Overhulser, 1992; Mustart et al., 1995; Verdú and García-Fayos, 1998). Traveset (1993a) found a negative correlation between the number of parthenocarpic fruits in Pistacia terebinthus and the number of wasp-damaged seeds, suggesting that empty fruits can serve to lower predation risks. Ziv and Bronstein (1996) showed that moths avoid infertile seeds by flying away from trees where they find them thus reducing the impact on viable seeds.
Zangerl et al. (1991) reported that butterfly larvae (Depressaria pastinacella) preferred the parthenocarpic fruits of Pastinaca sativa (Umbelliferae) due to the lower content of furanocoumarins (toxin) compared with seeded fruits.

The purpose of the present work was to determine the breeding system of Bursera morelensis, describing pollination and fruiting ecology, and following the maturation of seeds and fruits from the anatomic and histological perspective to determine the presence of parthenocarpic fruits or apomictic seeds in the plant, and its relationship with seed predation by insects.

MATERIALS AND METHODS

Study site

The study site was located near Barranca de Muchil in San Rafael Coxcatlán, in the southeast portion of the Tehuacan Valley, Puebla, México (18°12’ and 18°14’ N; 97°07’ and 97°09’ W), at 1000 m a.s.l. and has a dry climate with summer rains (Fernández, 1999). Mean annual temperature is 25 °C and mean annual rainfall 394.6 mm, with a dry season from November to May and a rainy season from June to October (Valiente, 1991). It is an alluvial fan where the predominant vegetation is tropical deciduous forest with 57 species of angiosperms reported (Fernández, 1999). It has a high soil diversity (Medina, 2000) resulting in the formation of different vegetal associations: the ‘Fouquerial’ dominated by Fouqueria formosa Kunt, the ‘Cuajiotal’ dominated by Bursera morelensis Ramírez, the ‘Chiotillal’ dominated by Escontria chiotilla (Weber) Rose and ‘Cardonal’ dominated by Pachycereus weberi (Coulter) Buxb. (Ríos-Casanova et al., 2004).

Species studied

Bursera morelensis is a dioecious tree endemic to Mexico. Male flowers are produced in paniculated or inflorescent racemes while female flowers can be solitary, in pairs or in short panicles. Female flowers have non-functional anthers. Fruits are trivalvated ovoid (5–8 mm long; 4–6 mm wide). Seeds are covered by a yellow pseu-

Breeding system

Phenology was described by monthly visits to the study area from May 2005 to May 2007. Forty individuals were observed and flowers, fruits and leaf production was followed.

The breeding system was determined using six trees, where three branches were randomly chosen for each treatment; in each branch ten inflorescences were chosen for each pollination treatment (10 inflorescences × 3 branches × 3 treatments × 6 trees). The total number of inflorescences used was 540 and total flowers was 5930. The experiment was carried out in May 2006 and was followed until January 2007, and was done separately from the phenological observations. The number of fruits formed after pollination, number of full-grown fruits, and fruits with and without seed was registered in each treatment. All fruits produced in the experiment were collected and dissected. Three pollination treatments were applied.

1. Open pollination (control treatment: n = 2013 flowers). Inflorescences were marked and the number of flowers available counted. Flowers were exposed to biotic pollinators and abiotic factors. When they were dry, they were covered with mosquito mesh to avoid fruit loss.

2. Manual pollination (n = 1992 flowers). Flower buds were enclosed before anthesis. Flowers were hand-pollinated using pollen from 12 male trees. Each female flower received pollen from three different male trees. Flowers were enclosed after pollination and fruit production was monitored.

3. Pollination exclusion (n = 1925 flowers). Flower buds were enclosed with fine mosquito mesh and flowers left open were enclosed for the duration of their lives. Fruit production was followed.

Fruit development

Of the fruits formed in May 2005 and May 2006, 50 fruits were collected from 14 randomly chosen plants of Bursera morelensis in April 2006 and in April 2007, respectively, 11 months after flowering when they reached full size and maturity. Fruits collected in this part were independent of those of the pollination experiment. Total dimensions (width and length in millimetres), weight (dry and fresh in grams), colour and presence of odour (Berlanga, 1991; Martínez, 1996) were measured. External features were measured using an electronic caliper with a resolution of 0.01 mm. Fresh weight was measured using an analytical balance while the dry weight was obtained using a dryer to 70 °C until the sample reached a constant weight. Fruit type was noted (with or without seed).

Monthly collections of different stages of fruit formation were carried out to follow fruit development using anatomical techniques (López et al., 2005). Fifty fruits each month were collected between June 2006 and February 2007 from three randomly chosen trees. Fruits were collected and fixed in FAA (formol: acetic acid: 96 % ethanol: water, 1 : 0·5 : 5 : 3·5) and transported to the laboratory where they were embedded in paraffin and stained with safranin–fast green. Also collected were flowers which were embedded in LR-White and stained with toluidine blue. Fruits were dissected to describe all developmental stages. Fruits were classified according to their characteristics. Parts of the fruits were dried to critical point, coated with gold and observed under the scanning electron microscope (SEM).

Insect seed predation

The proportion of damaged seeds was determined, collecting 50 fruits from 23 randomly chosen trees.
Fruits were dissected and classified as fruits with or without seeds and damaged or undamaged. Insect-damaged fruits were those containing eggs or larvae inside or those presenting a hole by which the insect abandoned the fruit after hatching. The proportion of damaged and empty fruits was calculated and a correlation between variables was carried out.

To identify parasitic insects, 70 fruits were collected, isolated inside plastic bags and placed in total darkness until fruit maturation and the insects hatched. The insects were collected and preserved in 70% alcohol for further identification by a specialist. To determine the timing of infection, 50 fruits were collected monthly from three randomly chosen trees and dissected to search for eggs or larvae. Fruits were collected in April 2007 when the fruits produced in May 2006 were maturing.

**Results**

**Phenology and breeding system**

Flowering in *B. morelensis* was synchronic and occurred in the two observed years 1 week following the first rains (third week of May 2005 and second week of May 2006). Male buds formed earlier than female buds and anthesis followed the same pattern. Male flowers lasted between 5 d and 7 d while female flowers lasted between 3 d and 4 d. Pollination was completed by bees (*Apis mellifera*).

Anthers were present in the female flowers of *B. morelensis* but they were not developed and did not produce pollen.

Flowering lasted 2 weeks and after that immature fruits could be seen in trees. Immature fruits were green and reached their full size (7–8 mm) in 1 week. Maturation time was between 7 and 8 months with a maximum of 11 months (Table 1). Fruits became red when maturing.

Pollination experiments showed that there were two important times of fruit loss, the first from flower to fruit

| TABLE 1. Phenology of *Bursera* morelensis for the 2 years of observations (May 2005 to May 2007) in the Tehuacan Valley, México. Light shading indicates the presence of flowers, leaves and immature fruits; dark shading indicates the presence of mature fruits |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May |
| 2005 | | | | | | | | | | | | |
| Flowering | | | | | | | | | | | | |
| Presence of leaves | | | | | | | | | | | | |
| Fruiting | | | | | | | | | | | | |
| 2006 | | | | | | | | | | | | |
| Flowering | | | | | | | | | | | | |
| Presence of leaves | | | | | | | | | | | | |
| Fruiting | | | | | | | | | | | | |
| 2007 | | | | | | | | | | | | |
| Flowering | | | | | | | | | | | | |
| Presence of leaves | | | | | | | | | | | | |
| Fruiting | | | | | | | | | | | | |
formed completely, and the seed itself was trigonous surrounding the seed coat completely. The seed coat was valves through these lines. The pseudoarile was orange dehiscence. Fruit opened completely separating three occurring (Fig. 1).

The plant is self-incompatible and no pollen limitation is 1.54 n% of fruit set (Fig. 1). In the open pollination treatments, 63.59 ± 17.31 % of the flowers formed fruits (n = 2013 flowers) and 46.62 ± 21.18 % of these reached full size. Most of those fruits had seeds (42.47 ± 21.47 %) while the others had no seeds but presented two different types of tissues inside (2.23 ± 2.77 %). Hand-pollinated treatments resulted in 59.34 ± 16.97 % of fruit set (n = 1992 flowers) and 38.91 ± 14.60 % of full-sized fruits formed. Almost all these fruits formed seeds with only 2.73 ± 1.98 % being seedless. In pollen-exclusion treatments only 2.26 ± 4.01 % of fruit set (n = 1925 flowers), and of these only 1.54 ± 2.99 % grew to full size and none contained seeds. The plant is self-incompatible and no pollen limitation is occurring (Fig. 1).

**Fruit development**

Overall, four different fruit types in *Bursera morelensis* were found (Fig. 2 and Table 2; n = 700 fruits). Fruit size was not different either in the total length or width, nor for fresh or dry weight (P > 0.05).

Type I fruit was red in colour and had three lines of dehiscence. Fruit opened completely separating three valves through these lines. The pseudoarile was orange surrounding the seed coat completely. The seed coat was formed completely, and the seed itself was trigonous measuring 5.7 ± 0.7 mm in length and 5 ± 0.3 mm in width. Seed colour was grey dotted with black.

Type II fruits were also red, with the three dehiscence lines but, when ripening, dehiscence was incomplete in 100 % of cases. From a total of 280 type II fruits, only 191 were opened by one valve (68.2 %), 78 opened by two valves (27.85 %) and 11 (3.92 %) presented dehiscence in at least one valve but this was never complete. In the areas without dehiscence, the pseudoarile was not formed; in the areas with dehiscence the pseudoarile was orange. When some part of the seed with pseudoarile became exposed the exocarp dehydrated and the pseudoarile became brown. Seed measurements were 5.4 ± 0.6 mm in length and 4.8 ± 0.5 mm in width. Seeds were white dotted with black spots. The seed coat was not completely formed and presented two types of tissue: one hard and white with the other soft and translucent. The only way to separate fruits of type I and II in the tree was by observing valve dehiscence.

Fruit types III and IV were similar. They were green to red depending on maturation stage. Fruit tissues were not differentiated and no dehiscence formed. Type III presented one to two ovules and type IV presented a tissue instead of ovules in the locules.

Inside type III fruits three locules, each bearing two ovules, were found. When the fruit was growing (approx. 4–5 mm in diameter) only one locule persisted with one of the two ovules being aborted and the other developing into a seed. Usually, there was one seed per fruit. The ovary walls were well defined (Fig. 3). Inside type IV fruits all the locules were occupied by tissue with obliterated locules, ovary walls were diffuse, and ovules had either degenerated or were absent (Fig. 3). Type IV fruits represented parthenocarpic fruits and could be differentiated under a microscope from the first month after blooming.

In type III fruits, ovary layers showed a different arrangement than type IV fruit (Fig. 4). The tissue found filling the locule in type IV fruits was formed by cells from endocarp and mesocarp (Becerril, 2004) that grew into the locule squashing the ovule (Fig. 4). In the seeded fruits (type III) the mesocarp and endocarp were formed by cells with relatively thick walls and calcium oxalate crystals. In the parthenocarpic fruits, cells were larger with thin walls and calcium oxalate crystals were absent (n = 21 fruits randomly chosen) (Fig. 5).

Type III fruit had small ovules that remained small for 5–8 months, after which they reached full size when they dehisced and were ready for seed dispersal. Type III fruits were classified as such when immature; however, upon reaching full size they became type I (Figs 3 and 6). Similarly, type IV fruit represented the immature phases of type II.

The outermost layer that protected the seed was a tissue derived from the endocarp. In the seeded fruits it was a continuous layer that protected the seed, but in the parthenocarpic fruits the layer was incomplete.

**Insect seed predation**

Of the fruits examined, 79.15± 2.79 % (range 43.6– 98.2 %) presented seeds, 17.6 ± 2.2 % (range 1.75–45.45 %)
were parthenocarpic and 3.05 ± 0.89% (range 0–18%) were parthenocarpic and parasitized by flies of the family Cecidomyiidae (Diptera) and wasps of the superfamily Chalcidoidea (Hymenoptera; \( n = 1150 \) fruits, 23 trees, 50 fruits per tree). Insects began laying eggs on fruits in the seventh month of fruit development. Dissected fruits for the observation of fruit development showed also that insects visit the fruits in the seventh month of development. It was not possible to determine hatching time. Only one larva per fruit was recorded and only in parthenocarpic fruits. Parasitized fruits could be easily recognized because the puncture that the insect made to insert eggs in fruits was full of resin forming a white scar. Of the 280 parthenocarpic fruits dissected 62 were parasitized, while of the 870 fruits with seeds examined none was parasitized. A negative and significant correlation between the percentage of damaged fruits and the percentage of parthenocarpic fruits in a tree was found (\( r^2 = -0.71, \) d.f. = 22, \( P < 0.05 \); Fig. 7).

**DISCUSSION**

**Breeding system**

Flowering of *B. morelensis* occurred after the first summer rains in May. Flowering synchrony can be a strategy to avoid competition for pollinators (Frankie et al., 1974). This phenological pattern has been reported for several species of the tropical dry forests such as *Plumeria rubra* and *Guazuma ulmifolia* (Borchert et al., 2004). The delay of female flower maturation related to male flowers is a frequent pattern between dioecious trees (Lloyd and...
Webb, 1977; Obeso, 1996), and has been related to the higher energetic costs of female related to male plants (Lovett-Doust and Lovett-Doust, 1988).

Fruit set reported here for *B. morelensis* was similar to values reported by Jordano (1988) in the three pollination treatments and slightly lower that those reported by Verdú.

![Fig. 5](Image) Mesocarp of a seeded fruit and a parthenocarpic fruit. Calcium oxalate crystals, indicated by an arrow on the left, are absent on the right. Scale bar = 10 µm. Micrographs were taken with phase contrast under an optic microscope (Olympus Provis AX70).

![Fig. 6](Image) Developmental stages of seeded (A–C) and parthenocarpic (D–F) fruits of *Bursera morelensis*. From left to right upper line, growing ovule (A), embryo in development (B), seed without a valve (C). In the lower line, fruit full of tissue with a developing ovule (D), fruit full of tissue squashing ovule (E), parthenocarpic fruit as can be seen in trees (F). a, Ovule; b, ovary wall; c, locule; d, embryo. Scale bar = 1 mm; Macrographs were taken with a stereoscopic microscope (Zeiss).
and García-Fayos (1998) for *P. lentiscus*. Fruit production is limited by pollen availability as shown by the high abortion rate of enclosed flowers as stated by other authors (Jordano, 1988; Verdú and García-Fayos, 1998; Bañuelos and Obeso, 2005). Pollination can be affected by the number of pollinators available, number of visits and distance between trees of different sex in dioecious species (Pascarella, 1996; Bañuelos and Obeso, 2003; de Jong et al., 2005). Abortion can also be a consequence of environmental scarcity of resources, seeds being genetically deficient or seed predation and damage (Janzen, 1971, 1977; Stephenson, 1981; Aker, 1982; Ehrlén, 1991; Bañuelos and Obeso, 2005).

Seeds produced by *B. morelensis* clearly had a sexual origin. This if often related to the maintenance of high variability in the population that can ensure adaptation in the long term (Muller, 1964; Michod and Levin, 1988; Kondrashov, 1994; Hurst and Peck, 1996; Doncaster et al., 2000; Maynard-Smith and Szathmáry, 2001; Rice and Chippindale, 2001).

**Fruit description and development**

Parthenocarpic fruits in *B. morelensis* can be recognized and separated from seeded fruits in trees when maturation has finished because of the incomplete dehiscence of the valves; however, during the long period in which fruit are immature, these two types cannot be distinguished as is the case in many other angiosperms (Jordano, 1988; Traveset, 1993b; Fuentes, 1995).

There are many reports of plants producing parthenocarpic fruits but most of them involve an induced phenomena to produce seedless fruits for commercial purposes (Varoquaux et al., 2000; Ampomah-Dwamena et al., 2002; Zohary, 2004). In species where parthenocarpy is believed to exist naturally, there are no complete anatomical descriptions of fruit development. In *P. lentiscus*, parthenocarpic fruits presented only remnants of the funicule without any trace of embryos (Jordano, 1988), and in *P. terebinthus* the fruits presented remnants of both funicule and ovules (Traveset, 1993a). In *B. morelensis* traces of funicule and ovule were observed but a huge spread of probably mesocarp and endocarp was also found. This suggests that different factors (environmental, genetic, physiological) may promote the production of parthenocarpic fruits in the different species studied (Gillaspy et al., 1993).

Parthenocarpy can originate by several factors both internal and external. Sources of external factors include scarcity of resources (Bertin, 1995; Jang and Sheen, 1997; Sato et al., 2001), thermal stress (Sato et al., 2001, 2002; Higashiya et al., 2003; Young et al., 2004), hydric stress (Gay et al., 1987) and damages in the reproductive organs (Galil and Eisikowitch, 1971; Solomon, 1980). Internal causes include changes in hormone concentration (Nitsch, 1950; Gillaspy et al., 1993; Azcon-Bieto and Talon, 2000; Fos et al., 2003), polyploidy and errors in gene expression (Mazzucato et al., 1998, 2003; Varoquaux et al., 2000; Ampomah-Dwamena et al., 2002; Carmi et al., 2003; Zohari, 2004).

**Parthenocarpy and insect seed predation**

Seed predation registered for *Bursera morelensis* (3.05 ± 0.89 %) was lower than that registered in other parthenocarpic species such as *Olea europaea* (18 % ± 8.8 %; Jordano, 1987), *Pastinaca sativa* (14.4 %; Zangerl et al., 1991), *Pistacia lentiscus* (0.4–2.9 %; Jordano, 1989; Verdú and García-Fayos, 1998) and *P. terebinthhus* (9 %; Traveset, 1993a). This could probably be related to the presence of resins that characterise *Bursera* and decrease survivorship and growth rates in some larvae (Becerra, 1994). It is probable that insects have evolved some mechanism enabling them to parasitize *Bursera* fruits, with smaller survivorship and growth rates, such as that described for beetles feeding on leaves of *Bursera* (Becerra and Venable, 1990; Becerra, 1994; Evans et al., 2000). According to Traveset (1993a), it is possible that insects can differentiate between fruits with and without ovules while inserting the ovipositor. If this is true, the growth of soft tissue inside parthenocarpic fruits of *B. morelensis* might serve to deceive parasitizing insects.

The pollination experiment showed that 2 % of the fruits produced were parthenocarpic and without parasites, nevertheless when the number of parasitized fruits was determined it was found that approx. 20 % of the fruits were parthenocarpic and 3 % had insects. In the pollination experiment, fruits were protected by the bags preventing the entrance of insects, while in the other experiment the fruits developed without protection. The presence of parasites and the increase in the number of parthenocarpic fruits could suggest that insects could be one of the factors that promote the formation of parthenocarpic fruits as indicated by Galil and Eisikowitch (1971) and Solomon (1980), although further studies are necessary to investigate it.

Parthenocarpy has been proposed to be related to seed predation in *Pastinaca sativa* (Zangerl et al., 1991). The hardness of the fruit wall depends on the internal layer of mesocarp, which is full of crystals of calcium oxalate, and the lignified endocarp. In parthenocarpic fruit when the mesocarp spreads and the cell became more elonged, the endocarp gets fragmented forming unprotected sites.
The presence of crystals has been regarded as a defence against seed predation (Franceschi and Horner, 1980; Sunell and Healey, 1985; Perera et al., 1990; Ward et al., 1997; Webb, 1999; Molano-Flores, 2001; Ruiz et al., 2002). The production of calcium oxalate crystals is a specialized process analogous to bone formation in animals (Webb, 1999). These crystals are produced as a way of metabolizing harmful elements such as oxalic acid (Franceschi and Horner, 1980; Carvalhão, 1997), and serve to store calcium, minimizing the amount of calcium in circulation but maintaining calcium available for tissue formation (Franceschi and Horner, 1980; Tilton and Hornet, 1980; Fink, 1991; Webb, 1999), and minimize predation (Molano-Flores, 2001). Chemically they are considered as irritating compounds that reduce palatability and for some insects are toxic (Smith, 1989). Absence of insects in the fruits with crystals of calcium oxalate of B. morelensis suggests that the plant can produce fruits armed mechanically and chemically to ensure seed development as suggested by Lee et al. (1991), and enhance an attraction unit for seed dispersers (Traveset, 1993a; Fuentes, 1995; Fuentes and Schupp, 1998; Verdú and García-Fayos, 1998). Nevertheless more studies are needed to assess the effect of the presence of calcium oxalate crystals on parasitization and survival rates of insects (Smith, 1989; Zangerl et al., 1991; Molano-Flores, 2001).

Parthenocarpy was reported previously for B. morelensis and B. fagaroides by Verdú and García Fayos (1998), and during the present work this type of fruit was observed in B. aptera, B. schlechtendalli and B. submontiflora. Recently, Bonfil et al. (2007) reported parthenocarpy in B. grandifolia, B. bipinnata, B. lancifolia, B. copallifera, B. glabrifolia and B. bicolor. This suggests that parthenocarpy is a widespread phenomenon in the genus Bursera but more studies are needed to demonstrate this. This adds weight to the proposal of Verdú and García-Fayos (1998) that parthenocarpy was present in a common ancestor of the families Anacardiaceae and Burseraceae, and this character was positively selected for some reason and remains today. Although the original function of parthenocarpy is still unknown, presently it is proposed that parthenocarpic fruits can enhance attraction to seed dispersers and lower the individual probability of a seeded fruit being parasitized (Zangerl et al., 1991; Traveset, 1993a, b; Fuentes, 1995; Ziv and Bronstein, 1996; Fuentes and Schupp, 1998; Verdú and García-Fayos, 1998; M. F. Ramos-Ordóñez, unpubl. res.).

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LITERATURE CITED


