

Floral movements have been appointed as mechanisms to avoid self-pollination (Darwin, 1862), and new evidence indicates that they reduce intrafloral male-female interference as well (Barrett, 2003; Sun *et al.*, 2007). Motion occurs in different floral parts: corolla, gynoecium, style, stigma, or stamens, and motility is either insect-induced or active (Ruan and Teixeira da Silva, 2011). A recent review on adaptive advantages of floral movement indicated four main benefits: (1) they promote outcrossing, (2) allow delayed selfing, (3) support tolerance to harsh environments, and (4) reduce intrafloral male-female interference (Ruan and Teixeira da Silva, 2011). Investigation of motility of floral elements and its adaptive significance allows understanding evolution of mating systems, ecological habitat shifts and pollinator environments in different groups of plants (Herlihy and Eckert, 2002; Kalisz *et al.*, 2004; Goodwillie *et al.*, 2005; Barrett, 2008). Stamen movement has been documented in a few plant families, among them Berberidaceae (Lechowski and Bialczyk, 1992). Ren (2010) suggested that there are four main types of stamen movement: cascade movement (Weigend and Gottschling, 2006); quick and explosive movement (Taylor *et al.*, 2006); simultaneous and slow movement (Azuma *et al.*, 2005); and stimulated movement (Lechowski and Bialczyk, 1992; Lebuhn and Anderson, 1994). Stamen movement can affect the reproductive biology of flowering plants because this mechanism forces the visitors to enter flowers and may alter the interaction plant-pollinator (Schlindwein and Wittmann, 1997; Edwards *et al.*, 2005; Liu *et al.*, 2006; Ren, 2010).

In the case of Berberidaceae, stamen stimulated movement has been associated with a quick transmembrane flux of ions and subsequent water efflux (Bialczyk and Lechowski, 1988; Lechowski and Bialczyk, 1992). It is usually triggered by insects that visit

and touch stamens, enhancing the probability of the anther/pollen coming into contact with pollinators, or for helping the anther come into contact with a self pistil, promoting autogamy (Ren, 2010).

Here we selected *Berberis trifoliolata*, a shrub known by common names like agarito, algerita, chiper and palo amarillo to corroborate if a stimulated movement of stamens occurs in response to contact with visitors as previously reported for Berberidaceae (Lechowski and Bialczyk, 1992) as well as to identify pollinators and floral visitors. Plants of *B. trifoliolata* are small to medium sized, evergreen shrubs that produce abundant red berries. Agarito is one of the noteworthy species in the arid and semi-arid lands of North America, in the Chihuahuan and Sonoran Deserts and in Tamaulipan thorn scrub.

We conducted fieldwork in two localities, in the foothills of the Cerro Potosí in Galeana, Nuevo León, a mountain located in northeastern Mexico in the Chihuahuan Desert (24° 53' 16" N and 100° 15' 12" W). Altitude is approximately 2,100 m a.s.l. and vegetation is thorny semi-

arid scrub. The second study site was located within the Mezquital Valley, a semiarid shrubland at the north of Ixmiquilpan on the southern area of "Cerro La Muñeca" in Hidalgo (20° 37' 12.8" N and 99° 13' 44.8" W). Altitude is approximately 2,129 m a.s.l. (Figure 1). Observations of floral visitors were made during the flowering peak in March of 2011 and 2012. The behaviour of bees visiting the flowers was observed. To estimate floral visitors a transect method was followed (revised in Dafni *et al.*, 2005) using three transects measuring 10 × 50 m along which the frequency, time of occurrence and visit rate were recorded. Each individual within a transect was observed for a 10 minute period from 0700 to 1430 h over six days in 2011, and from 0700 to 1700 h over three days in 2012. Insects were killed with ethyl acetate for identification and deposited in the entomological collection of the Instituto de Ecología, A.C. (IEXA).

Our field observations detected a sweet smell emitted by the corollas throughout anthesis. The number of showy yellow flowers varies from 1-4 per raceme, and plants usually produce two close racemes. Fruit are red

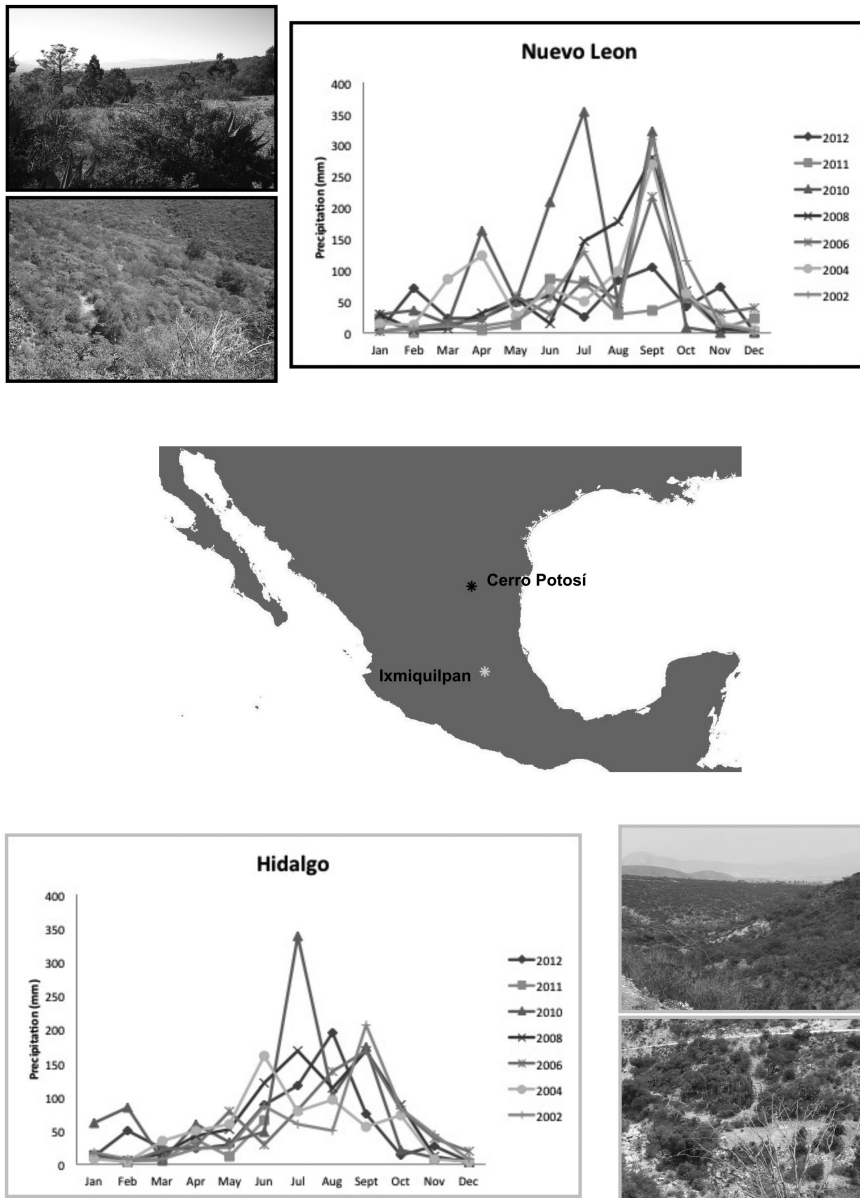
## FLORAL MOVEMENTS: STAMEN MOTION IN *BERBERIS TRIFOLIOLATA*

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**Figure 1.** Location of Cerro Potosí, Nuevo León and Ixmiquilpan, Hidalgo, where fieldwork was conducted. Images of Tamaulipan thorn scrub and semiarid shrubland at the Mezquital Valley are included. Precipitation for the last ten years are shown in the graph.

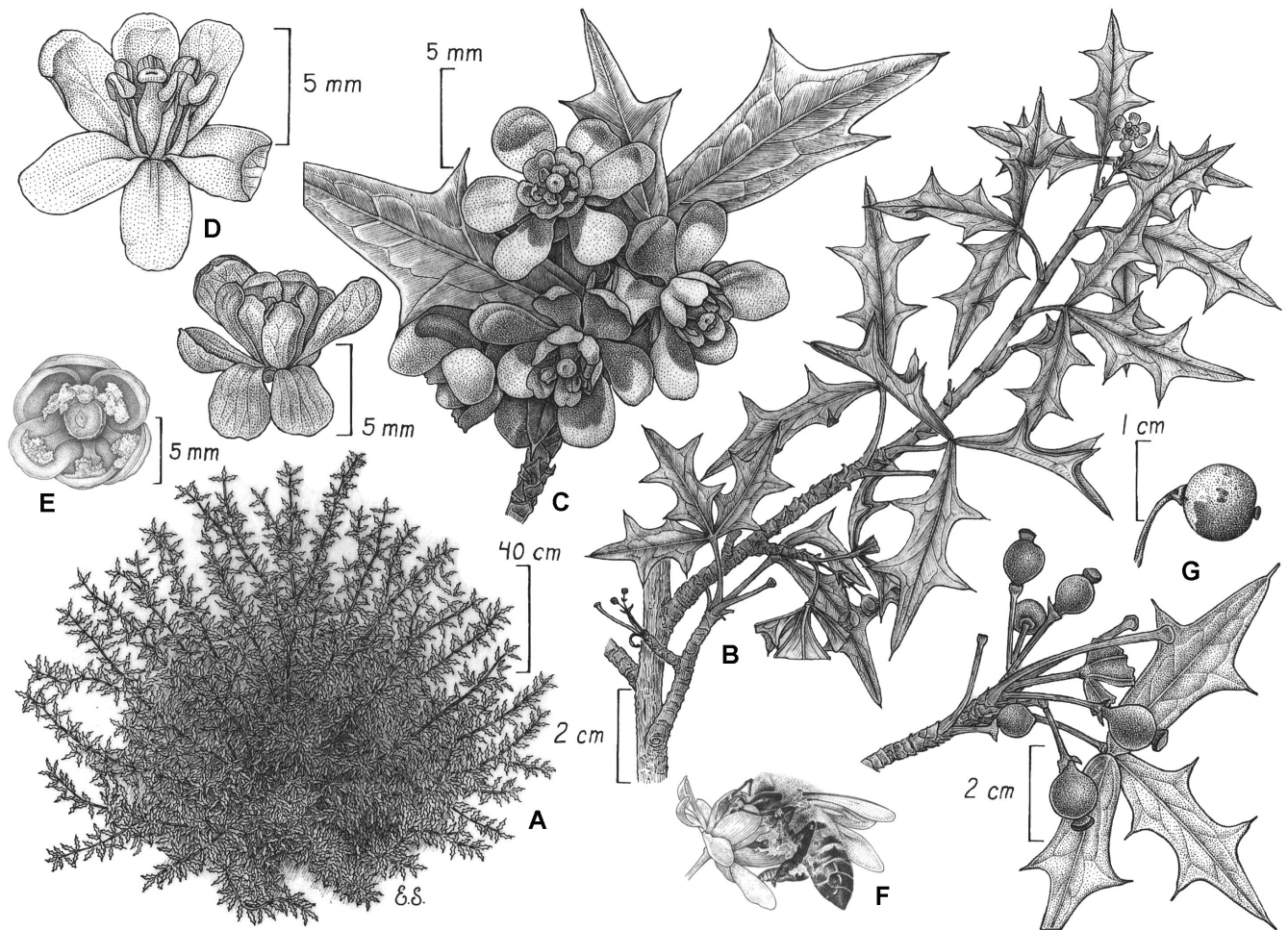
spherical berries that are fleshy and juicy with citrus flavor (Figure 2). Flowering season is in winter-spring. In both field study sites, blooming started in February, reaching a peak by mid-February and continued through early April, ending in late April and early May. Flowers open successively along inflorescences and anthesis last  $6 \pm 2$  days ( $n = 20$ ). At Cerro Potosí,

the number of flowers per individual was  $194 \pm 87.3$  ( $n=10$ ). At Ixmiquilpan, the number of flowers per individual was  $42.5 \pm 24.4$  ( $n = 8$ ).

In both field study sites, in newly opened flowers, the following tripping mechanism occurs: the stamens are pressed to the outside of the flower against the corolla. When the inner surface of the filaments is stimulated

by an insect visitor, the filament snaps inward, placing pollen from the anther on the vector. Later, the stamens return to their original position against the corolla. Nectaries are located on both sides of each of the six stamens. When they are touched by visitors probing for nectar, the filaments rapidly propel the anthers to the stigma, placing pollen on the body of the pollinator. Filaments can be folded independently forcing the pollinator to move around the flower and its legs to touch neighboring flowers, thus increasing the amount of pollen that adheres to the insect (Figure 2). In Cerro Potosí, Nuevo León, flower-visiting insects included members of Apidae, Syrphidae and Vespidae. The most common visitor was the honeybee, *Apis mellifera*. In addition, two species of flies (*Copestylum mexicanum* and *Copestylum* sp.), and a social wasp (*Vespula* sp.) occasionally visited flowers. Our observations in Ixmiquilpan, Hidalgo, detected other pollinators, like the bee *Megachile* sp. and plants are not pollinated by honeybees. Our field site in Cerro Potosí was surrounded by cornfields and beehives are abundant. Thus, we believe that the abundant populations of *Apis mellifera*, instead of native bees, pollinated the agarito flowers in this site. It has been discovered that honeybees dominate the pollinator assemblage in sites close to field crops (Sáez *et al.*, 2012).

The tripping pollination mechanism described above facilitates the transport of pollen by an insect that moves among the flowers of different plants, touching receptive stigmas with the pollen on the surface of its body, completing cross-pollination. Bees are important pollinators for other plant species of arid and desert regions in North America (Arizaga *et al.*, 2000; Clark-Tapia and Molina-Freaner, 2004; Pendleton *et al.*, 2008; Ortíz *et al.*, 2010). Previous research has found that they are the main pollinators in *Berberis*. Small Adrenidae, *Apis mellifera* and *Bombus* sp. have been reported for *B. thunbergii*



**Figure 2.** Habit, leaves, inflorescences, details of flowers and fruit of *Berberis trifoliolata*. A) habit, B) branch, C) inflorescence, D) flower before insect visitation, E) flower with anthers folded after insect visitation, F) honey bee visiting agarito flower, G) branching fruit and berry.

(Lebuhn and Anderson, 1994). Peterson (2003) reported that bees, wasps, flies, and beetles are the pollinators in *B. vulgaris*. Our results show *Apis mellifera* to be an effective pollinator for *B. trifoliolata*. Honeybees are known for moving short distances within the plant favoring transfer of pollen from one flower to another in the same individual (Schaffer *et al.*, 1983; Corbet, 1991).

It has been demonstrated that nectar production in plants from arid and semi-arid lands is influenced by a number of factors such as weather variables like daily maximum temperature and precipitation (Lloyd *et al.*, 2002) or demographic parameters

(Golubov *et al.*, 2004); nectar evaporates at high temperatures (Nocentini *et al.*, 2012). In the case of agarito, nectar was so scarce that we were unable to collect any amount. In Cerro Potosí precipitation was low and temperature was high compared to previous years (see Figure 1). Thus, further questions for study include whether *B. trifoliolata* also produces abundant nectar, if humidity or demographic parameters are correlated to its production and if an increase in the amount of nectar attracts more and native pollinators. Moreover, it has been demonstrated that native bees select flowers with abundant nectar, rejecting flowers

with scarce or without nectar (Aizen *et al.*, 2011).

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