A REASSESSMENT OF THE FUNCTION OF FLORAL NECTAR IN
Croton suberosus (Euphorbiaceae): A reward for
plant defenders and pollinators

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Typically, plant–pollinator interactions are recognized as mutualistic relationships. Flower visitors, however, can potentially play multiple roles. The floral nectar in Croton suberosus has been proposed to operate as a reward for predators, especially the wasp Polistes instabilis (Vespidae), which kills herbivorous insects, while the plant has been thought to be mainly wind-pollinated. In this study, we reassessed the pollination mode of C. suberosus and the possible role of its flower visitors. Pollinator exclusion experiments demonstrated that C. suberosus should be considered a strictly entomophilous species. Inflorescences of C. suberosus were visited by a diverse entomofauna involving 28 taxa belonging to six orders; however, wasps and bees were the only visitors that carried C. suberosus pollen. The visitation rate of wasps was approximately four times that of bees. This observation, combined with the fact that the small size of bees makes effective contact of their bodies with the stigma difficult, strongly suggests that large wasps are responsible for most of the effective pollination of C. suberosus. Among the wasp visitors, P. instabilis seems to be one of the most important. These findings expose an unusual plant–insect interaction, in which the plant provides nectar and wasps pollinate and defend the plant.

Key words: bout duration; Croton suberosus; Euphorbiaceae; Mexico; neotropical dry forest; plant–animal interaction; Polistes instabilis; pollen limitation; pollen load; pollination mode; Vespidae; visitation rate.

In plant–pollinator mutualistic interactions, nectar is produced in the flowers as a reward for pollinators, and thus plant pollination is assured (Pellmyr, 2002). Other mutualistic interactions are well documented in plants with extrafloral nectaries, in which extrafloral nectar is offered as a food source to predators or parasitoids that defend plants against herbivores (e.g., Janzen, 1966; Ness, 2006; Sugiu et al., 2006). Thus, extrafloral nectar is interpreted as a reward for defense against herbivores, whereas floral nectar is considered a reward for pollinators (Beattie and Hughes, 2002; Pellmyr, 2002). However, exceptions to this pattern are documented in multiple interaction systems: flower nectar can be consumed by plant defenders if the flowers have previously been damaged by nectar-robbing insects (Newman and Thomson, 2005) and flower nectar is opportunistically consumed by predatory ants that indirectly defend the plant against herbivores (Yano, 1994).

Croton suberosus Kunth (Euphorbiaceae), a monococious neotropical shrub, represents another unusual plant–insect interaction system; the wasp Polistes instabilis Saussure (Vespidae) defends C. suberosus foliage against herbivorous caterpillars, but the plant only produces flower nectar (Domínguez et al., 1989). Polistes instabilis seems to be highly effective in killing insects because levels of herbivore damage to the leaves are extremely low, although acceptability tests indicate that leaf tissue is readily palatable to some caterpillars and grasshoppers (Domínguez et al., 1989; Dirzo and E. Narbona, unpublished data). Because this plant species has been thought to be wind-pollinated (Domínguez et al., 1989), nectar was regarded as a reward for predatory wasps rather than for pollinators. However, pistillate and staminate flowers are visited by a variety of insects, and the relative contribution of wind pollination to overall pollination has not been assessed in detail. Available literature (Webster, 1994; Culley et al., 2002) has reported C. suberosus as an ambophophilous species (i.e., combining both biotic and wind pollination). The pollination system of the large genus Croton is poorly known, but it is expected to be diverse because entomophily (mainly Hymenoptera, Diptera, and Coleoptera), anemophily, and ambophily have been documented in the genus (Bullock, 1994; Webster, 1994 and references therein; Armbruster et al., 1999; Williams and Adam, 1999; Freitas et al., 2001).

Croton suberosus has floral traits consistent with anemophily (sensu Culley et al., 2002), including unisexual flowers, a reduced perianth and long stigmas, but it also has traits consistent with insect pollination, including floral nectaries and the presence of pollenkitt (E. Narbona and R. Dirzo, personal observation). However, some of the floral traits associated with anemophily could also be attributed to wasp pollination (Faegri and van der Pijl, 1979) or could simply represent a phylogenetic constraint in floral design (Johnson and Steiner, 2000).

The role of P. instabilis as a defender against foliar herbivores in C. suberosus is well established, but its role in pollination has
not been quantified in spite of its importance as a floral visitor (Domínguez et al., 1989). The main objective of this study was to investigate the pollination system of *C. suberosus* and to clarify the roles of *P. instabilis* and other floral visitors. Specifically, we examined the following questions: (1) What is the pollination mode of *C. suberosus* (wind, biotic pollination, or both)? (2) What is the spectrum of flower-visiting insects associated with *C. suberosus*, and does it differ between staminate and pistillate flowers? (3) Do floral visitors to *C. suberosus* transport its pollen, and if so, which taxa transport greater pollen loads?

**Materials and Methods**

**Species and study site—** *Croton suberosus* is a deciduous shrub occurring in seasonally dry tropical forests of the south Pacific coast of Mexico, typically growing in disturbed conditions of such forests (Rzedowski, 1978; Domínguez et al., 1989). The plant produces its leaves during the rainy season, just before flowering time (July to August). Individuals are monococious and usually have a basal branch that produces successive secondary branches, and in the apex of each branch, a dense racemose inflorescence develops. Racemes have an acropetal trend when flowering, with pistillate flowers situated at the base and staminate flowers at the apex. Each raceme produces ca. 20 pistillate flowers and ca. 40 staminate flowers (E. Narbona and R. Dirzo, unpublished data). Inflorescence flowering duration is about 5 d for the pistillate phase and 17 d for the staminate phase (Domínguez and Bullock, 1989). Overlapping of sexual phases of inflorescence is extremely rare (Domínguez and Bullock, 1989) (Fig. 1), but flowering synchrony of flowers of the same individual is not perfect; thus, geitonogamy may be possible because the species is self-compatible (Domínguez and Bullock, 1989). Pistillate flowers are apetalous and have three large bidental styles (Fig. 1A, B). A receptive stigmatic surface is located only at the end of each style branch (Fig. 1A, B). Styles are straight and stigmas are sticky and green when pistillate flowers are receptive (Fig. 1A), whereas styles become curved and stigmas are dry and brown when flowers are not receptive (Fig. 1B, C). Staminate flowers have five white petals (Fig. 1C, D), and their 15 stamens are clustered into a single column at the center of the flower. The column is flanked by a basal branch that produces successive secondary branches, and in the apex of each branch, a dense racemose inflorescence develops. Racemes have an acropetal trend when flowering, with pistillate flowers situated at the base and staminate flowers at the apex. Each raceme produces ca. 20 pistillate flowers and ca. 40 staminate flowers (E. Narbona and R. Dirzo, unpublished data). Both types of flowers produce nectar because they have floral nectaries situated between sepalas and petals in staminate flowers and between the sepalas and the base of the styles in pistillate flowers. Pistillate and staminate flowers produce a volume of nectar of 5.1 ± 1.8 (mean ± SE) and 6.8 ± 2.3 μL/flower, respectively (E. Narbona and R. Dirzo, unpublished data). Daily nectar secretion occurs between 0800 and 1600 hours (Domínguez et al., 1989). Fruits are three-seeded capsules with explosive dispersal that mature ca. 20 d after fertilization. Ants have been seen carrying fruits, likely performing secondary dispersal.

This study was carried out at the Chamela Biological Station, located in the State of Jalisco, western Mexico (19°30′N, 105°03′W). Mean annual precipitation is 852 mm with most of the rains concentrated between June and October (García-Oliva et al., 2002). The predominant vegetation is seasonally dry tropical forest (Rzedowski, 1978). We studied one population of about 400 individuals situated in a sunny area along the borders of a main road leading to the station (150 m a.s.l.).

**Pollination mode and pollen limitation—** To assess the pollination mode and the possible transfer of pollen from staminate to pistillate flowers in the same *C. suberosus* inflorescence, we randomly selected 73 individuals at the beginning of the flowering period (July) in 2006. Prior to anthesis of the pistillate flowers, inflorescences were covered with a bag that was tied around the base of the peduncle. Due to the low number of inflorescences produced by most plants, we used only one inflorescence per individual. Each inflorescence was randomly assigned to one of the following treatments: “apomixis” (the production of seed without sexual reproduction, including pseudogamy), which was tested using pollen-proof paper bags; “anemogamy,” tested using small-mesh polyester bags (pore size ca. 0.8 × 0.8 mm), which were permeable to airborne pollen (Neal and Anderson, 2004); and “entomophily by small insects,” which was assessed using an optical microscope. Pollen grain counts on the body of the insects were determined using an optical microscope. Pollen grain counts on the body of the insects were based on pollen that originated only from *C. suberosus*. Voucher specimens of all floral visitors are retained at the Chamela Biological Station, Universidad Nacional Autónoma de México.

**Flower visitor activity and analysis of pollen loads—** To assess quantitative aspects of pollination, we censused flower visitors during 4 d of the flowering peak in 2006 (2 d each for plants with either staminate-phase or pistillate-phase inflorescences). On each census day, we selected six individuals with only one flowering inflorescence, in the pistillate or staminate phase. The number of flowers in anthesis per inflorescence ranged from six to eight. Random censuses of 15-min duration were made of the selected plants from 0900 to 1800 hours, collecting the overall activity of floral visitor taxa (Domínguez et al., 1989; E. Narbona, personal observation). The total number of censuses was 36 for pistillate-phase inflorescences and 32 for staminate-phase inflorescences. During each census, we recorded (1) the insect species, (2) the number of visits of each taxon to the inflorescence, (3) the duration of each visit, and (4) the behavior of insects (i.e., nectar vs. pollen searching, parts of the flower contacted, and predatory behavior). We recorded “visitation rate” as the number of visits per 15 min observation period (Fishbein and Venable, 1996), and “bout duration” as the time spent foraging on an inflorescence by an insect (Potts, 2005). We studied inflorescences rather than flowers because both pistillate and staminate flowers generally act as a unit from the point of view of anthesis synchrony (Domínguez and Bullock, 1989; E. Narbona, personal observation) and due to the difficulty in differentiating isolated flowers within inflorescences (Fig. 1).

The total number of flower visitor species of a plant may be frequently underestimated as a result of poor sampling effort (Herrera, 2005). To assess control for sampling effort and gauge any possible biases regarding flower visitor diversity, we used sample-based rarefaction curves (i.e., species accumulation curves; Colwell et al., 2004). This method is used to estimate species richness in an area, but can be readily applied to assess floral visitor assemblages by considering samplings instead of sampling plots and inflorescence visits instead of individuals (see detailed methods in Herrera, 2005). To estimate the true number of species of flower visitors, we used the incidence-based coverage estimator (ICE; Colwell, 2006). Percentages of collected species were assessed by dividing the observed species by the ICE (Watkins et al., 2006). Rarefaction curves and diversity estimators were separately computed for staminate and pistillate inflorescences by performing 100 randomizations. We employed rarefaction curves scaled by inflorescence visits because we are interested in analyzing the species richness of visitors to inflorescences (see Colwell et al., 2004).

Discrimination between true pollinators and flower visitors was assessed by analyzing the body pollen loads (Young, 1988). Flower visitors were captured haphazardly from *C. suberosus* inflorescences during the 3 d of the flowering peak; attempts were made to include representative specimens of all taxa that had previously been recorded as floral visitors. To avoid contamination, we captured small and medium insects with a vial and transferred them to a freezer (Bernhardt, 2005); butterflies and some wasps were captured individually with a net, killed in a killing jar, and then transferred to a vial. We measured the “total vector pollen load” (sensu Inouye et al., 1994) as the total number of pollen grains on the body of an insect, irrespective of position and without regard to the probability of successful deposition of pollen onto a stigma. Because pollen grains are of sufficient size, pollen loads were counted directly on the insect body using a dissecting microscope (20× magnification; Fig. 1). The vials were checked for pollen grains that may have detached from the insects, but none were found. Pollen grains packed in the specialized pollen-carrying organ (metasomal sternum) of *Asmunea viella* sp. (Megachilidae) were not considered (Talavera et al., 2001). Prior to analysis of pollen loads, pollen grains of *C. suberosus* and other species comprising intact inflorescences were determined using an optical microscope. Pollen grain counts on the body of the insects were based on pollen that originated only from *C. suberosus*. Voucher specimens of all floral visitors are retained at the Chamela Biological Station, Universidad Nacional Autónoma de México.

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Fig. 1. Insect visitors and aspects of (A, B) pistillate-phase and (C, D) staminate-phase inflorescences of *Croton suberosus*. (A) *Polybia* sp. sucking nectar on a pistillate flower (white arrow indicates receptive stigma contacting the ventral surface of the insect). (B) Pistillate flowers with a brown-colored stigmatic surface (because anthesis is finished); note that stigmatic surface only represents a small proportion of the total length of the style. (C) *Polistes instabilis* searching for nectar on staminate flowers; note that several pollen grains have adhered to the ventral part of the insect body. (D) Three coleopteran species eating pollen of staminate flowers.
DATA ANALYSIS—Fruit set and seed set for the pollination mode and the pollen limitation experiments were explored by generalized linear models (GLM) with probit link function and quasi-binomial error structure (McCullagh and Nelder, 1989). We used quasi-binomial fit instead of binomial to correct data overdispersion (Crawley, 2005) applying an F test for analysis of deviance, performed with software R version 2.5.0 (R Development Core Team, 2007). Because analyzing the overall variation between all treatments is not biologically meaningful, we made the comparison by pairs of treatments biologically relevant (e.g., anemogenesis vs. open). To control for experimentwise type I error produced by multiple comparisons, we applied the sequential Bonferroni correction for fitting the significance level (García, 2004).

Prior to analyzing data of insect visitors, we explored the link function that was best adapted for our data, using the smaller deviance model (McCullagh and Nelder, 1989). Differences in visitation rate and bout duration of insect visitors were tested by means of GLMs, assuming Poisson error distribution and the power link function. Explanatory variables tested were: groups of insects, sexual phase of the inflorescence, and their interaction. Pearson $\chi^2$ was used to correct for overdispersion of data (StatSoft, 2001). Analyses were carried out using the GLZ module in the program STATISTICA 6.0 (StatSoft, 2001). Preliminary observations suggest that wasps and bees may play an important role in pollination process; thus we compared visitation rates and bout durations between both groups by means of a priori contrasts using the GLM procedure. Again, we applied sequential Bonferroni corrections (García, 2004). Differences in pollen loads between insect groups were tested using a one-way ANOVA with type III sum of squares due to unbalanced sample size (Shaw and Mitchell-Olds, 1993).

We employed the EstimateS software version 8.2 (Colwell, 2006) to calculate rarefaction curves and diversity estimators. To compare diversity of flower visitors between both types of inflorescences, we used rarefaction curves, which were considered significantly different if and when the 95% confidence intervals did not overlap (Colwell et al., 2004).

RESULTS

POLLINATION MODE AND POLLEN LIMITATION—Fruit set of control inflorescences ranged from 11.1 to 100%, with an average of 66.4% (Fig. 2). In the apomixis and anemogamy treatments, bagged flowers did not produce any fruit (Fig. 2), suggesting that isolated pistillate flowers of C. suberosus need biotic vectors for pollination. Only 3.8% of flowers in the small insects treatment developed fruit (Fig. 2), and this fruit set was markedly lower than in the control treatment ($F_{1,10} = 25.54, P < 0.00001$). Selfing within inflorescences in the absence of biotic vectors was possible but improbable (Fig. 2), and thus the within-inflorescence nonmanipulated geitonogamy and within-inflorescence anemogamy treatments had a significantly lower fruit set than the control treatment ($F_{1,24} = 59.17, P < 0.00001$ and $F_{1,20} = 38.15, P < 0.00001$, respectively). An average of 14.8% of inflorescences in the within-inflorescence small insects treatment developed fruit (Fig. 2), which was significantly lower than in the control treatment ($F_{1,23} = 29.52, P < 0.0001$). Species of the Chrysomelidae, and Ashmeadiella sp. (Megachilidae) and Crematogaster sp. (Formicidae) were found inside some bags in the small-mesh bag treatments. Seed set in this treatment was not significantly different from that of the control treatment (66.6% and 70.6%, respectively; $F_{1,12} = 0.758, P = 0.40$).

Inflorescences in the pollen supply treatment did not develop significantly more fruits than those in the control treatment ($F_{1,28} = 2.70, P = 0.111$; Fig. 2). Average seed set in the pollen supply treatment was 74.1%, which was not significantly different from that of the control treatment ($F_{1,22} = 0.096, P = 0.76$).

FLOWER VISITOR ACTIVITY AND ANALYSIS OF POLLEN LOADS—Inflorescences of C. suberosus were visited by 28 insect taxa belonging to six orders (Table 1). Pistillate-phase inflorescences received visits from 19 taxa, and staminate-phase inflorescences were visited by 27 taxa. Our sampling effort accounted for a considerable proportion of the total species diversity; the observed insect visitor richness was 78% and 83% of the estimated true richness, based on the ICE estimator for pistillate- and staminate-phase inflorescences, respectively. Although pistillate-phase inflorescences were less visited than staminate-phase inflorescences, the diversity of insect visitors in both phases was statistically indistinguishable, as judged by the overlap of the confidence intervals of both rarefaction curves (see Appendix S1 in Supplemental Data with online version of this article). Reduviidae (Hemiptera) and Membracidae (Homoptera) were casual visitors (one visit only) and were not included in the calculation of visitation rates or bout durations.

Total visitation rate for staminate-phase inflorescences was nearly double that of pistillate-phase inflorescences ($1.14 \pm 0.11$ vs. $0.58 \pm 0.11$; mean $\pm$ SE), and the difference was highly significant ($\chi^2 = 14.3, P < 0.0001$). Visitation rates differed significantly among insect groups ($\chi^2 = 65.2, P < 0.00001$; Fig. 3A), but differences among insect groups were not homogeneous when considering stamine- and pistillate-phase inflorescences (i.e., there was a significant inflorescence phase x insect group interaction; $\chi^2 = 43.8, P < 0.00001$; Fig. 3A). For pistillate-phase inflorescences, ants were the most abundant visitors, followed by lepidopterans and wasps, whereas for stamine-phase inflorescences, the most abundant visitors were coleopterans, followed by wasps (Fig. 3A). Wasp hives made consistently more visits to flowers than bees in both sexual phases of the inflorescences, and these differences are highly significant ($\chi^2 = 12.7, P < 0.0001$ for the pistillate phase; $\chi^2 = 16.4, P < 0.00001$ for the staminate phase; Fig. 3A). Among wasps, the most frequent visitors to pistillate-phase inflorescences were P. instabilis (45%) and Eumenes spp. (30%), whereas Eumenes spp. were the most frequent visitors (31%) to staminate-phase inflorescences, followed by P. instabilis (18%) (Table 1). Among bees, Trigona fulviventris was the most frequent visitor to pistillate-phase inflorescences (67%), and Ashmeadiella sp. was the most frequent visitor (95%) to staminate-phase inflorescences (Table 1).
Overall, insect taxa bout durations were not statistically different between pistillate-phase and staminate-phase inflorescences (204 s ± 26.4 vs. 251 s ± 22.3; Wald $\chi^2 = 0.42; P = 0.52$). Bout durations differed significantly among insect groups (Wald $\chi^2 = 31.1, P < 0.00001$; Fig. 3B), but there was no significant interaction between inflorescence phase and insect group (Wald $\chi^2 = 5.09, P = 0.40$; Fig. 3B). Coleopterans and ants had the longest visits to both pistillate- and staminate-phase inflorescences (Fig. 3B). Wasps and bees had mean bout durations shorter than 52 s, and bout duration was not significantly different between these groups for either pistillate-phase (Wald $\chi^2 = 0.78; P = 0.78$) or staminate-phase inflorescences (Wald $\chi^2 = 0.04, P = 0.83$; Fig. 3B).

All wasps were observed feeding on nectar (Table 1; Fig. 1A, C). Bees generally searched for nectar (Fig. 1B), but in some cases Ashmeadiella spp. were observed collecting pollen (packed in metasomal externa). Ants sought nectar, although Pseudomyrmex sp. and Camponotus sp. were only observed walking on the inflorescences. Coleopterans were active in eating pollen (Fig. 1D), although small Cantharidae and Tenebrionidae also searched for nectar. Lepidopterans and dipterans only consumed nectar. All insect visitors that gathered nectar took it from the nectaries at the base of sepals (Fig. 1A–D). Members of the Reduviidae were observed predating on bees and small coleopterans.

Only three of the eight floral visitor insect groups (wasps, bees, and coleopterans) transported pollen, although among coleopterans the presence of pollen was trivial (<1 grain per individual, Table 1). The average number of pollen grains carried by wasps and bees was 52.6 (coefficient of variation, $CV = 121.7$) and 61.4 ($CV = 84.7$), respectively, but high variation was found among samples (Table 1), and consequently, differences between these insect groups were not significant ($F_{1, 22} = 0.08, P = 0.78$). Pollen was present in all taxa of wasps and bees, but the wasps P. instabilis, Brachysphaera sp., and Eumeninae sp. 1, and the bee Ashmeadiella sp. had the highest pollen loads on their bodies (an average of 80 or more grains per individual; Table 1).

For wasps, sticky pollen was deposited mainly on the ventral surface of some part of their bodies when they gathered nectar from staminate flowers and was subsequently transferred to stigmas during nectar-seeking in pistillate flowers (Table 1; Fig. 1A, C). The large size of wasps (body lengths of all taxa, except Eumeninae sp. > 15 mm) enabled them to touch the stigma
infl or.: inflorescence, COLE: Coleoptera, LEPI: Lepidoptera, DIPT: Diptera. Insect groups included taxa shown in Table 1.

effective pollination difficult because the styles are longer than (Fig. 1A). Both recorded bees are smaller than 8 mm, making with their ventral surface, presumably resulting in pollination (Fig. 1A). Both recorded bees are smaller than 8 mm, making effective pollination difficult because the styles are longer than their bodies.

DISCUSSION

Our results show that C. suberosus relies entirely on pollinators for reproduction because bagged pistillate flowers did not produce seeds when insect pollinators were excluded. This result was expected because the presence of pollenkits renders difficult the dispersion of pollen by wind, and sticky pollen is a typical feature of entomophilous taxa (Culley et al., 2002; Pacini and Hesse, 2005). Although a very improbable event, selfing within an inflorescence in the absence of biotic vectors was found in C. suberosus. It is known that complete interfloral dichogamy (i.e., no flowering overlap between pistillate and staminate flowers) is effective in avoiding geitonogamy within inflorescences (Bertin and Newman, 1993; Aluri et al., 1998). Croton suberosus has a high degree of, but not complete, inter-floral dichogamy (Domínguez and Bullock, 1989; E. Narbona, personal observation), and thus it is possible for intrainflorescence pollination to occur if pollen grains of staminate flowers fall onto the stigma of pistillate flowers. However, anthesis of pistillate flowers of bagged inflorescences is much longer than that of unbagged inflorescences (E. Narbona, personal observation) because flowers that are effectively pollinated rapidly become senescent (Primack, 1985). Thus, natural intrainflorescence pollination of C. suberosus in the absence of biotic vectors is a highly unlikely event.

Croton suberosus is apparently pollinated by medium-sized and large insects because small insects (with body diameters less than approximately 2 mm) contributed little to the overall fruit set of the plant. However, entire inflorescences covered with mesh bags produced a relatively higher fruit set than those with the staminate part removed (14.8% vs. 3.8%), suggesting that the movement of small insects throughout the inflorescences can result in some geitonogamous pollination. This experiment may have overestimated the relative pollination by small insects because, as noted above, the probability of anthesis overlapping for pistillate and staminate flowers in unbagged inflorescences is very low. Furthermore, the mesh bags provide an artificial environment in which small insects can forage undisturbed by larger, aggressive ones (Keys et al., 1996), and indeed we observed that most of the insects that entered the mesh bags did not leave the inflorescences until the bags were removed.

The inflorescences of C. suberosus were visited by a taxonomically diverse array of insects. The most abundant visitors were species of the order Hymenoptera, followed by Coleoptera and Lepidoptera. The insects were very diverse in morphology and fed mainly on nectar, although a few fed on pollen. Such a spectrum of insects contrasts somewhat with that observed on other Croton species, which have been reported to be visited mainly by dipterans and hymenopterans (Reddi and Reddi, 1985; Armbruster et al., 1999). Nectar-bearing flowers in species of this genus are typically open to insects, including those with mouth parts unspecialized for nectar feeding. In C. suberosus and C. sarcopetalus (Freitas et al., 2001), the nectariferous flowers are not completely open to insects because the floral nectaries are partially covered by the sepal petals and the petals in staminate flowers and by the sepal petals and the base of the styles in pistillate flowers (Fig. 1). Thus, these structures must be forced aside when insects, such as hymenopterans, insert their proboscides for nectar gathering (Faegri and van der Pijl, 1979; Aluri et al., 1998), although some lepidopterans can insert their long, slender proboscides between the flower parts to obtain nectar (Faegri and van der Pijl, 1979).

Insect groups varied in their visitation rates and in the rates of visitation between sexual phases of the inflorescence. Staminate-phase inflorescences were visited almost twice as frequently as those of the pistillate phase, a difference that can be attributed to the greater preference of coleopterans, wasps, and bees for staminate-phase inflorescences. Coleopterans mainly collect pollen, and it was thus expected that they would make more visits to staminate-phase inflorescences. Because wasps only feed on nectar and bees search for both nectar and pollen, the fact that staminate flowers produce almost twice as much nectar as pistillate flowers (Domínguez et al., 1989) provides a possible explanation for this differential preference between flower sexual phases. In addition, staminate flowers have five white petals, which may help to attract insects (Arista and Ortiz, 2007).

![Fig. 3](image-url)
Pollination effectiveness can be considered in qualitative and quantitative terms (Herrera, 1987, 1989). Assessment of pollination quality requires knowledge of the number of pollen grains deposited on the stigma during a single visit and the preferences and movement patterns of pollinators (Herrera, 1987; Johnson and Steiner, 2000). However, in measuring the quantity component and analyzing pollen loads in relation to insect morphology and behavior, we were able to infer differential pollination effectiveness (Herrera, 1987; Fishbein and Venable, 1996). In the case of *C. suberosus*, we were somewhat surprised that frequent flower visitors with long residence times, including lepidopterans, ants, and coleopterans, carried little or no pollen. We observed that coleopterans ate a large amount of pollen, while some ant species had aggressive behavior that discouraged effective pollinators; both groups could reduce the pollination effectiveness of other flower visitors, and consequently they may result antagonistic to *C. suberosus* (Ashman and King, 2005; Larsson, 2005; Ness, 2006). Wasps and bees were the only flower visitors that transported appreciable amounts of pollen, and thus we conclude that *C. suberosus* must be pollinated solely by these insects. Although wasps and bees transported similar numbers of pollen grains and had visits of the same duration, the visitation rate for wasps was approximately four times greater than that of bees for each of the two inflorescence sexual phases, and this could have a substantial influence on the pollination process (Gómez and Zamora, 1999; Sahl and Conner, 2007). Furthermore, bees appeared not to be efficient pollinators of *C. suberosus* because their small to medium size makes it difficult for them to touch the stigmatic surface of the pistillate flowers, as proposed for other species (Faegri and van der Pijl, 1979; Wilson et al., 2004). Although the role of the bees as pollinators could not definitively be excluded, all of the described factors suggest that effective pollination is, to a large extent, due to large wasps. Thus, *C. suberosus* could be considered an ecological generalist in that it interacts with a range of different insects, but a functional specialist in terms of effective pollination (Fenster et al., 2004; Wilson et al., 2004; Larsson, 2005). Similar specializations among the functional group of large wasps have recently been reported in other species (Shuttleworth and Johnson, 2006, 2009; Johnson et al., 2007). However, exclusion experiments that unequivocally permitted the definition of pollen loads delivered on stigmas by wasps and bees are needed to elucidate their definitive role as pollinators of plants such as *C. suberosus*.

The possibility that several wasp species pollinate *C. suberosus* may be important in ensuring its pollination success independent of fluctuations in pollinator populations in space and time (Fishbein and Venable, 1996; Waser et al., 1996). The evolution and maintenance of a multispecies pollination system is possible when different flower visitors have similar pollination effectiveness (Gómez and Zamora, 1999). Similar pollination effectiveness is expected in plants having flowers with few ovules (Johnson et al., 1995), as occurs in *C. suberosus* (three ovules per flower). Having few ovules per flower in combination with a high abundance and diversity of pollinators may decrease the probability of pollen limitation for plant reproduction (Liu and Koptur, 2003; Ashman et al., 2004; but see Gómez et al., 2007) and may explain why the Chamela population of *C. suberosus* was not pollen-limited in the study year.

Wasp species such as *Eumenes* sp., *P. instabilis*, *Brachygastra* sp., and species of the Eumeninae were the more frequent wasp visitors to inflorescences of *C. suberosus*. The latter three vespid species are probably the major contributors to the reproductive success of *C. suberosus* because they were the wasps that transported the largest quantities of pollen. All *Polistes* spp. and several vespid wasps feed on nectar, but they also predate on lepidopteran larvae as food for their own developing larvae (Faegri and van der Pijl, 1979; Raveraet Richter, 2000). *Polistes instabilis* is attracted to the flowers of *C. suberosus*, but also preys on foliar herbivores and defends the plant (Dominguez et al., 1989); in so doing, these species form a double mutualistic interaction. By providing evidence that *C. suberosus* produces nectar to reward pollinators, among which *P. instabilis* is one of the most important, our study complements previous work showing the role of these wasps as defenders of the plant and becomes the first study to report a plant–insect mutualistic interaction in which the plant provides nectar and the insect defends the plant and takes part in its pollination.

In conclusion, large wasps seem to be specialized pollinators of *C. suberosus*, although the role of bees and other medium-sized insects could not definitively be excluded. The flower traits of *C. suberosus* may have evolved in response to selective pressures exerted by functional interactions with these wasps (Fenster et al., 2004). In addition, this specialization may be reinforced or maintained if vespid species, including *P. instabilis*, affect the plant’s fitness via protection against foliar herbivores. Further studies are needed to evaluate the relative importance of each pollinator, including *P. instabilis*, on plant reproductive success.

**LITERATURE CITED**


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