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Short communication

# Frequency of visits by ants and their effectiveness as pollinators of *Condalia microphylla* Cav.

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ABSTRACT

The effect of a pollinator species on a plant depends on their interaction frequency and the pollinator's effectiveness. The role of ants as pollinators is highly variable, in some cases they act as true pollinators and in others as antagonists damaging flowers, robbing nectar or disrupting pollination. Pollinator visitation frequency has been demonstrated as the most important factor determining their impact on plants. Ants are commonly seen as visitors of the desert shrub *Condalia microphylla*, but their effectiveness as pollinators is unknown. In this study we assess the quantitative and qualitative role of ants and other winged flower visitors as pollinators of *C. microphylla* by experimentally quantifying their contribution to fruit production. The study was conducted in the Monte Desert of Villavicencio Nature Reserve, Mendoza, Argentina. A diverse assemblage of insects visited flowers of *C. microphylla*, including bees, ants, flies, beetles and vespids. Ants (*Camponotus mus* and *Camponotus punctulatus*) accounted for a high proportion of interactions. Fruit set resulted mostly from pollination by winged insects, while flowers visited by ants did not set fruits. Thus, although ants were commonly seen on flowers, their effectiveness as pollinator was negligible for *Condalia microphylla*.



## 1. Introduction

In mutualistic interactions, the impact of a species on another has two components, the frequency of interactions and the per interaction effect (Vázquez et al., 2012). Particularly in plant pollinator interactions, the effect of a pollinator on a plant depends on the effectiveness of that species (a measure of the quality of the interaction) and on the frequency of that interaction (Herrera, 1989; Vázquez et al., 2012). Effectiveness refers to the quantity of viable pollen that pollinators transfer to the stigma per visit, while frequency of interaction refers to the number of times each pollinator visits a flower of the species. It has been recently reported that the frequency of interaction is relatively more important for the fitness of the species than the per visit effect (the effectiveness) (Vázquez et al., 2012). This statement assumes that as the frequency of visits augments, the pollination function increases. However, it has been shown that for some species of plants as the number of visits increases, the pollination function stabilizes, or even might decrease (eg. for *Capparis atamisquea* in Morris et al., 2010). Ants are commonly seen as floral visitors, thus they are expected to exert a strong influence on the plants they visit and can represent a good framework to analyze the relative contribution of effectiveness and frequency of visits.

There is growing evidence that ants can be true pollinators for many species as they are common visitors on flowers and are able to carry pollen that results in seed set (e.g. de Vega et al., 2009; Gómez and Zamora, 1992; Kawakita and Kato, 2002). The named ant pollination syndrome includes plants that have a high density of very small flowers bearing overlapping inflorescences at a uniform height, and low seed number, pollen volume and nectar quality and this syndrome is particularly common in hot, dry habitats (Hickman, 1974). But, ants visiting flowers are usually considered as non-pollinating insects performing antagonistic effects on plants by damaging floral resources, thieving the nectar, disrupting pollination and limiting reproductive processes (Dutton and Frederickson, 2012; Galen and Butchart, 2003; Ghazoul, 2001; Hull and Beattie, 1988). It seems that their effectiveness in the transfer of viable pollen can be especially important for this group of frequent visitors to flowers, but the examination of the role of

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ants on the reproduction of plants has not often been assessed (Ashman and King, 2005).

The purpose of this study was to assess the quantitative and qualitative role of ants and other flower visitors on *Condalia microphylla* by experimentally analyzing the contribution of ants and other winged insects to fruit production, in a plant that has many characteristics of the named ant pollination syndrome. We expected that if the effect of the frequency of visits was strong, the number of visits by each group would be positively related to the fruit set, otherwise the visitation rate would be independent of the fruit set, indicating that the quality effect (effectiveness) was important for the species.

## 2. Materials and methods

*C. microphylla* Cav. (Rhamnaceae), is an evergreen xerophytic shrub endemic of South America, Argentina (Tortosa, 1999). Enthomophilous flowers are yellowish to greenish, small, bisexual, (4 or) 5-merous, in axilar cyme inflorescences. Disk is intrastaminal, nectariferous, fleshy and tightly surrounding the ovary which is superior. The species has many of the typical "ant pollination syndrome" (small, open flowers, readily accessible nectaries, low number of ovules and is very common in desert areas in Argentina) (Hickman, 1974). Fruits are fleshy drupes containing one seed with oily albumen and a large embryo. Seed dispersal is performed by birds or frugivorous mammals.

The species was sampled in the Monte Desert of Villavicencio Natural reserve located in Mendoza province, Argentina, where it blooms in spring and peaks between mid-October and mid-November. Bloom is consistent with the flowering peak of most species in this biome (Chacoff et al., 2012). This plant species is one of the core species of the plant–pollinator network as is one of the most generalized species, is not only highly visited but is also visited by many insect species (Chacoff et al., 2012).

We performed censuses of visit frequencies to *C. microphylla* shrubs in full bloom (i.e., >50% of their flowers opened) to determine the assemblage of visitors. We sampled visitors to flowers in 5 consecutive years (from 2006 to 2010). Censuses consisted of 5 min of observation to a flowering branch, during the census we recorded the number of open flowers observed and the number of flowers visited by each visitor (for details on the sampling procedure see Chacoff et al., 2012). We conducted 106 censuses during the five years, with a total of nearly 9 h of observation to flowering individuals from 7 am in the morning until 15 pm in the afternoon. In each observational day, we performed censuses to different plants that were located over 2 ha plots, over the 5 years of sampling some plants were measured more than once. For visitors that were not identified in the field we collected individuals for determination in the laboratory by taxonomists (see acknowledgments). We estimated the visitation rate for each visitor species as the average number of flowers visited per census (5 min). The abundance was the number of individuals observed on flowers. To quantify the importance of each visitor, we multiplied the abundance of each visitor by its visitation rate (following Herrera, 1989). In order to compare the role of ants and other winged insects, visitor's species were classified in two different groups: Ants and winged insects (flies, bees, vespids and others hymenopterans) and compared them by using the non-parametric Wilcox test. For this analysis we excluded beetles, as they were not commonly seen in *C. microphylla* flowers (Table 1).

To analyze the role of ants and other flower visitors in reproduction we experimentally excluded ants and winged insects from flowers. We randomly selected focal plants ($n = 10$ in 2008 and $n = 15$ in 2009). In each plant we applied four treatments. (1) "Open pollination" in which flowers were left to open pollination and all type of visitors were able to visit the flowers (2) "Ants pollination" in which flowers were visited only by ants; in these plants we covered inflorescences with mesh-bags that excluded all winged

**Table 1**
Visitation rate, abundance and the quantity of interaction of the insects that visit *Condalia microphylla* flowers.

| Order | Family (number of species) | Visitation rate (Visit flower$^{-1}$ h$^{-1}$) | Abundance (number of individuals) | Quantity of interaction (Individuals flower$^{-1}$ h$^{-1}$) |
|---|---|---|---|---|
| Hymenoptera (ants) | Formicidae (2) | 0.027 | 132 | 1.730 |
| Hymenoptera (others) | Apidae (1) | 0.037 | 31 | 1.140 |
| | Colletidae (3) | 0.004 | 3 | 0.004 |
| | Crabronidae (1) | 0.01 | 1 | 0.014 |
| | Eumenidae (2) | 0.003 | 3 | 0.006 |
| | Halictidae (2) | 0.004 | 16 | 0.033 |
| | Ichneumonidae (1) | 0.015 | 1 | 0.015 |
| | Megachilidae (1) | 0.0001 | 1 | 0.0001 |
| | Pompilidae (2) | 0.010 | 2 | 0.01 |
| | Sphecidae (1) | 0.001 | 4 | 0.01 |
| | Thynnidae (1) | 0.009 | 6 | 0.052 |
| | Vespidae (2) | 0.003 | 5 | 0.007 |
| Diptera (flies) | Agromyzidae (1) | 0.0001 | 1 | 0.0001 |
| | Asilidae (2) | 0.001 | 8 | 0.005 |
| | Bombyliidae (7) | 0.004 | 146 | 0.059 |
| | Calliphoridae (1) | 0.013 | 40 | 0.536 |
| | Empididae (2) | 0.01 | 2 | 0.007 |
| | Muscidae (1) | 0.005 | 3 | 0.014 |
| | Nemestrinidae (1) | 0.01 | 68 | 0.91 |
| | Sarcophagidae (1) | 0.002 | 1 | 0.002 |
| | Sciomyzidae (1) | 0.010 | 1 | 0.010 |
| | Syrphidae (8) | 0.01 | 59 | 0.054 |
| | Tachinidae (4) | 0.004 | 12 | 0.033 |
| | Tephritidae (1) | 0.002 | 3 | 0.006 |
| | Therevidae (1) | 0.014 | 1 | 0.014 |
| Coleoptera (beetles) | Chrysomelidae (1) | 0.004 | 18 | 0.066 |
| | Coccinellidae (1) | 0.003 | 10 | 0.03 |
| | Nitidulidae (1) | 0.010 | 1 | 0.010 |

insects but allowed ants to crawl up the stems and visit flowers. (3)“Winged pollination” in which flowers were visited only by winged insects that can reach the flowers because of flying. Ants were excluded by the application of glue (Tanglefoot), lubricating oil and also by covering with cotton the base of the stems. Moreover, to avoid that ants could walk to the flowers by stepping on other branches we pruned branches that contacted the focal inflorescence or branch. (4) “No pollinators” in which flowers could not be visited by any insects because they were inside a mesh bag along the flowering period, and by the application of glue and cotton at the base of the branch stems. Each pollination treatment had 2 replicates per plant (two branches per treatment) and was replicated over 25 plants. When setting the experiment we counted the number of floral buds and at the end of ripening we counted the number of fruits formed in the focal branch. Fruit set was calculated as the proportion of flowers that set fruits, and preferred instead of pollen deposition as a proxy for the estimation of female reproductive success for each treatment (Ne’eman et al., 2010). Although we did not measure directly the per interaction effect, that is, how many fruits are formed by only one visit of each pollinator; we assessed the influence of both, quantity and quality of pollination performed by each group. We used a general linear model that considered the condition of pollination (open, ants, winged and no pollinators) as predictor and fruit set as the response variable in R (R Development Core Team, 2011). Data reported throughout the text, tables and figures are mean values ± 1SD.

## 3. Results

*C. mycrophylla* flowers were visited by 53 insect species, belonging to 28 families (31 species of flies, 17 species of hymenopteran that were bees, vespids and wasps, two species of ants and tree species of beetles, Table 1). In total, considering the five years only 0.3% of the observed flowers received a visit (3099 visits in 1,100,920 observed flowers, Table 1). Visitation rate varied greatly among species and groups (Table 1) being Apidae the family with the highest visit rate (the only species in this family was *Apis mellifera*), followed by ants. Bombylid flies were the most abundant group visiting flowers followed by ants, which had the highest quantity of interactions (Table 1). Ants that visited flowers were identified as *Camponotus mus* and *Camponotus punctulatus* and visited 222 and 165 flowers, respectively. Both species visited flowers at similar rate (0.017 ± 0.08 and 0.032 ± 0.025 visits per flower/5 min of observation respectively) and they had also similar abundance (79 and 53 individuals seen respectively) on flowers.

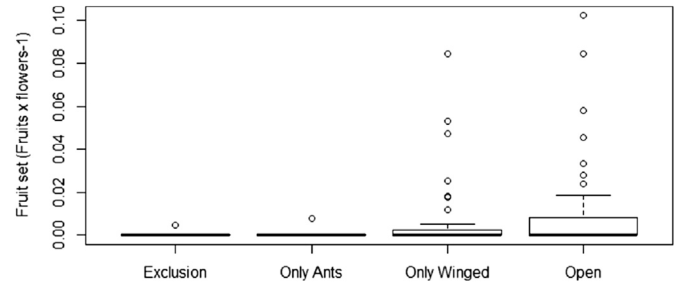


**Fig. 2.** Fruit set of *C. microphylla* at Villavicencio Natural Reserve, Monte Desert from Argentina, under different pollination treatments. Boxplots must be interpreted as in Fig. 1.

When compared winged vs. ants, ants were more abundant in flowers (66 ± 18 vs. 8.7 ± 15, Wilcox test: $W = 94$, $p = 0.01$), visited flowers at a higher rate than winged insects (0.027 ± 0.007 vs. 0.005 ± 0.006, $W = 94$, $p = 0.02$) and thus the quantity of interactions was higher for ants than for winged (20.76 ± 0.45 vs. 0.96 ± 2.64, $W = 96$, $p = 0.01$) (Fig. 1).

Mean fruit set from open pollinated flowers was 0.012 ± 0.025 (ranged from 0 to 0.18), while the exclusion treatment set almost no fruits (0.0002 ± 0.0009) indicating that the plant species needed a vector to move the pollen from the anthers to the stigmas, thus it was animal pollinated.

Ants did not pollinate effectively flowers of *C. microphylla* as the production of fruits in either treatment (ant pollination and no pollinators) did not differ from zero (Estimated difference = 1.05, $z = 0.91$, $p = 0.7$, Fig. 2). Winged insects provided most of the pollination as fruit set from open pollinated flowers did not differ from those pollinated by winged insects (Estimated difference = 0.2574, $z = 2.007$, $p = 0.123$, Fig. 2).

## 4. Discussion

*C. microphylla* were visited by a diverse assemblage of insects that included bees, ants, flies, beetles and vespids, which resulted indispensable for fruit set, as flowers that did not receive visits did not set fruits. Although ants were the most important visitors in terms of their abundance in flowers and the time spent on them, they were not effectiveness as pollinators, as the flowers they visited did not result on fruit set. In this case, the effectiveness seems to be more important than the frequency of visits.

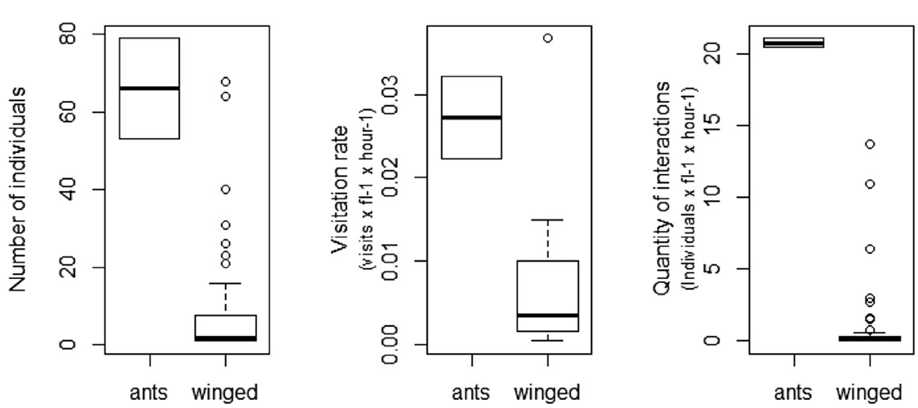


**Fig. 1.** Abundance, visitation rate and quantity of pollination performed to flowers of *C. microphylla* by ants and winged insects at Villavicencio Natural Reserve, Monte Desert from Argentina. All comparisons between ants and winged insects were statistically significant ($p < 0.05$). In each boxplot the horizontal line indicates the median; box limits are the first and third quartiles of the distribution; whiskers extend to the most extreme data point, which is no more than 1.5 times the inter quartile range; circles indicate outlying data points falling beyond whisker limits.

The relative abundance of ants with respect to the other species determines their role as true pollinators for some species, as ant pollination becomes evident when ants outnumber other floral visitors (e.g., Gómez et al., 1996). However, although ants represent one of the most abundant visitors on *C. microphylla*, they were not true pollinators for this species. Probably ants do not carry much pollen, and thus they became unimportant for the pollination or they are in the plant consuming the nectar from the extrafloral nectaries. Other possible explanation to the low effectiveness of ants as pollinators is that many ant species have metapleural secretions that can reduce the germination of the pollen grain (Beattie et al., 1984; Hull and Beattie, 1988). Although most species from the genus *Camponotus* do not have metapleural glands (Hölldobler and Engel-Siegel, 1984), there is evidence that this ant genus reduces pollen viability in some plant species (Beattie et al. 1985; Hull and Beattie, 1988). In some species, ants performed antagonistics interactions, by dissuading visitations of other pollinators (Tsuji et al., 2004; Willmer and Stone, 1997) or by thieving nectar of the flowers and damaging the pistils (Galen and Butchart, 2003). In the case of *C. mycrophylla*, ants can be robbers of nectar, thus their role can be antagonistic to the plant. We observed that many flying insects visited the flowers when ants were on them, thus they do not seem to have a dissuading role on this species. Further studies are needed to determine if ants can damage reproductive structures of the flower.

This study highlights the relative importance of the effectiveness component over the frequency as a determinant factor for the impact of ant visitors on flowers.

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