ISSN 1519-6984 (Print) ISSN 1678-4375 (Online)

How pollinator visits are affected by flower damage and ants presence in *Ipomoea carnea* subs. *fistulosa* (Martius and Choise) (Convolvulaceae)?

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Received: December 12, 2017 – Accepted: May 3, 2018 – Distributed: February 28, 2020 (With 3 figures)

Abstract

This study aimed to evaluate the effects of florivory and of the patrolling ants associated to EFNs-extrafloral nectaries, on the frequency of floral visitors, using the specie *Ipomoea carnea subs. fistulosa* (Martius and Choise) in Caatinga area. The floral attributes of the species were characterized. The effect of florivoria on the frequency of visitors and the influence of the presence of ants associated with the NEFs on the pollinator visit rate were evaluated. The rate of natural florivoria was recorded and collected floral visitors and ants over eight months. The damage on floral structure and the presence of ants foraging in the flowers causes a decrease in the number of total visits. The results may be justified by the fact that the floral damage consisted in the loss of important floral attributes. These effects for *Ipomoea carnea* subs. *fistulosa* can affect reproductive success, since it is a self-incompatible species and depends on the activity of the pollinators for their fertilization to occur.

Keywords: mutualism, antagonism, biotic defense, herbivory.

Como as visitas de polinizadores são afetadas por dano as flores e presença de formigas em *Ipomoea carnea* subs. *fistulosa* (Martius e Choise) (Convolvulaceae)?

Resumo

Este trabalho teve por objetivo avaliar o efeito da florivoria e do patrulhamento de formigas nos NEFs - nectários extraflorais sobre a frequência dos visitantes florais, utilizando a espécie *Ipomoea carnea* subs. *fistulosa* (Martius e Choise), em uma área de Caatinga. Os atributos florais da espécie foram caracterizados. Foram avaliados o efeito da florivoria sobre a frequência dos visitantes e a influência da presença de formigas associadas aos NEFs sobre a taxa de visita de polinizadores. Ao longo de oito meses foram registrados a taxa de florivoria natural foi registrada e realizada a coleta de visitantes florais e formigas. Os danos na estrutura floral e presença de formigas forrageando nas flores provocam decréscimo no número de visitas totais. Os resultados podem ser justificado pelo fato de que o dano floral consistiu na perda de atributos florais importantes. Estes efeitos para *Ipomoea carnea* subs. *fistulosa* podem afetar o sucesso reprodutivo, uma vez que é uma espécie autoincompatível e depende da atividade dos polinizadores para que a sua fecundação ocorra.

Palavras-chave: mutualismo, antagonismo, defesa biótica, herbivoria.

1. Introduction

The dynamics of the food web are strongly related to consumer-resource interactions. In this way, all forms of life are both Consumer and resource. The relationships of predator-prey types, herbivore-plant and parasite-host are classical examples of these interactions (Paine, 1980; Bronstein and Barbosa, 2002; Antiqueira and Romero, 2016). The interaction between flowers and pollinators is an important ecological process because it is an essential mechanism for cross-sexual reproduction to occur (Ashman et al., 2004). These interactions affect a wide variety of ecological and evolutionary processes (Ollerton, 1996; Ashman, 2004), and are crucial to the functioning of terrestrial ecosystems (Kevan, 1999). Among the most varied groups of pollinators, bees are the main suppliers of this service to angiosperms (Faegri and Van Der Pijl, 1979; Neff and Simpson, 1992), having a definitive role in plant reproduction. While herbivory plays an important role both in individual reproduction and in plant diversity, it is responsible for much of the plant richness and complexity of interactions in terrestrial systems (Ohgushi, 2005).

The effects of one species over another, mediated by a third species are termed indirect effects (Wootton, 1994) and are important for the structure and productivity of ecological communities and, therefore are widely studied by ecologists to elucidate their prevalence in nature (Rinehart et al., 2017; Castagneyrol et al., 2017).

The florivory, or herbivore damage to reproductive tissues prior to seed coat formation (Carper et al., 2016), is a type of interaction that occurs usually between plants and insects. It is associated with damage to structures with reproductive potential. The damage occurs from flower buds to flowers in anthesis (McCall and Irwin, 2006) can sometimes lead to nearly complete failure of seed set in some plant populations (Riba-Hernandez and Stoner, 2005).

There are two main ways, in which florivory can decrease plant fitness: florivores can destroy primary reproductive tissues, such as anthers, pistils, or ovaries, thus directly reducing gamete number (Althoff et al., 2005).

Numerous studies have shown that a large part of floral biomass is allocated to production of structures for the attraction of pollinators, such as petals and nectar (Valenta et al., 2017). Thus, when herbivores occurs in producing areas of resource (perfume, nectar, oils and resins), and even the anthers and stigma are damaged, the biotic quality of pollination tends to decrease, because the visual attraction becomes impaired (Canela and Sazima, 2003). It is known that several species of bees, the main group related to pollination, tend to avoid flowers, which present petals and / or stamens with damage (Krupnick and Weis, 1999).

In answer to the pressure of herbivory, plants have developed defense mechanisms (Mello and Silva-Filho, 2002). Among which, we can highlight the biotic defense associated with the production of food rewards, such as extrafloral nectaries (EFN), attracting mutualists partners (Heil and McKey, 2003). Predatory insects can visit the EFN, such as ants, that seek sugary secretions produced by them. Generally, foraging ants on the EFN is beneficial for plants because they prey herbivores that are present, contributing for prevention of possible damage to the plant and increasing their fitness (Koptur, 1994; Del-Claro et al., 2017).

However, although most studies address the benefits of association with predators to the plant, there are those who indicate otherwise, such as cases where pollinators and seed dispersors avoid flowers and fruit due to the presence of ants (Horvitz and Schemske, 1984; Almeida and Figueiredo, 2003).

The presence of predators (e.g. ants) in the flowers may be unfoavorable scenario for the floral visitors, inducing behavioral alterations in them (Belo, 2011). Moreover, considering the aggressive behavior of ants, associated with its carnivorous habit and the close foraging site of flowers, is possible that the patrolling ants attracted by the resources given to EFN can chase away potential pollinators (Heil and Mckey, 2003).

Both the impact of florivory, as ant's behavior on the flower visitors, is virtually unknown in temperate and tropical regions (Del-Claro and Torezan-Silingardi, 2009).

The present study aimed to test the following hypotheses using the plant model *Ipomoea carnea* subs. *fistulosa* to test the following hypotheses: H_1) Pollinators visited less damaged flowers; H_2) The patrolling ants associated with extrafloral nectaries (EFNs) reduces the number of pollinator visits.

2. Material and Methods

2.1. Study site

Field work was carried out from August of 2014 to March 2015, in the Seasonally Dry Tropical Forest (Caatinga Domain) at Tamanduá Farm, Santa Teresinha, State of Paraíba, northeast Brazil (7° 2' 20" S, 37° 26' 43" W). The Caatinga vegetation studied area consists of of shrubs and trees (Cabral et al., 2013) with additional herbaceous plants during the rainy season (Silva et al., 2012). Located in an average altitude of 240 meters, the soil type is shallow with low-fertility (Leptosoils) (EMBRAPA, 1997) with presence of rocky outcrops. The climate of the region is the BSh type of Köppen system, consisting of a dry and an irregular rainy season, with average annual rainfall around 600 mm. In 2014 and 2015, the cumulative rainfall levels up to April were 257 mm and 338 mm, respectively. The rainfall was monthly recorded, with a meteorological station installed in the study area.

2.2. Study system

The plant species used in this study was Ipomoea carnea subsp. fistulosa (Martius and Choisy) (Convolvulaceae), which is a perennial shrub, native to South America, and abundant in areas of Caatinga (Milet-Pinheiro and Schlindwein, 2005). The species bear ephemeral flowers, with diurnal anthesis (Maimoni-Rodella and Yanagizawa, 2007), self-incompatibility system which does not produce fruit even if submit to apomixis treatment or spontaneous self-pollinator and artificial self-pollinator, according Proctor et al. (1996). This species is pollinated by a variety of bees, such as Ancylocelis, Ceratina, Melitoma, Ptilothrix, Clementine, Apis and other (Kiill and Ranga, 2003). It attracts several species of ants, due to the presence of extrafloral nectaries: two located on the abaxial surface of the leaves, and five in the base of the sepals of flowers and buds (Paz et al., 2016). The EFN acording to Frey (1995) have defensive function against herbivores, but despite that, Keeler (1975) recorded a high rate of florivoria for this species in Bolivia and Costa Rica, Coleoptera Megacerus alternatus. We selected Ipomoea carnea subsp. fistulosa for this study by presenting a self-incompatibility system, interaction with the mandatory pollinators for their fertilization and extrafloral nectaries with patrolling ants but considerable rate of floral herbivory. The entire mentioned characteristics make the species *I. carnea* subs. *fistulosa* an excellent ecological model to evaluate the influence of florivoria and ants associated with extrafloral nectars on the visit of pollinators to flowers. *I. carnea* subs. *fistulosa* has branches and tangled roots forming a single population stain, hard to distinguish individuals. In this study, when necessary, the number of branches was used as sample unit.

2.3. Floral attributes and record of natural florivory

The corolla diameter was measured in 30 flowers. Flower size was classified according to Machado and Lopes (2004). The classification of floral form followed Faegri and Van Der Pijl (1979). In order to record the natural florivory and number of flowers and buds per inflorescence, an experiment was conducted, randomly collecting 6 clumps of different branches every hour from 5 am to 5 pm, totaling 78 inflorescences at the end of the experiment. The inflorescences were collected in paper bags with 10.5×25 cm. The number of insects present in buds and flowers, and fruit with some kind of damage was quantified.

2.4. Capture of floral visitors and ants visitors of extrafloral nectaries

During eight months (August 2014 to March 2015), species of flower visitors and ants were observed and captured manually using entomological net. The frequency of the species was calculated: F = number of months in which the species X was collected / total number of months collecting x 100. Species were classified according to Silveira Neto et al. (1976): X-constant> 50%, Y-accessory > 25-50% and Z-accidental <25%. The behavior and resource collected by the constant floral visitors were observed in 15 visits of each species.

2.5. Experiment I: florivory × visits of pollinators

Objective: Analise the rate of pollinator visits in damaged and intact flowers.

Procedure: 30 floral buds were isolated in 10 branches one day before anthesis, being a triade of buds on each branch. After anthesis, the flowers of each triade received treatments of mechanical damage using scissors: the first flower had loss 50% of the corolla while keeping the floral tube, the second flower had loss of 100% of the corolla, leaving only the reproductive organs exposed, and the third flower remained intact (Figure 1). The ants present at the inflorescences were eliminated, and the resin Tangle Foot[®] was applied to 20 cm of inflorescences to avoid interference of ants associated with extrafloral. This resin is non-toxic and not affect plant, acting as a physical barrier preventing access of ants to the plant (Del-Claro et al., 1996).

The experiment was repeated on three consecutive days to allow the observation and sampling of the three treatments simultaneously, totaling 30 flowers in each treatment (Freitas and Alves, 2008).

2.6. Experiment II: ants × visits of pollinators

Objective: Analise the influence of visitors' ants of extrafloral nectaries on pollinators

Procedure: 30 floral buds were isolated (5 control and 5 treatment) one day before the experiment. The ants were removed manually of each control branch and apllied the resin Tangle Foot[®]. We cut any branches of plants that could serve as bridge to ants acess the control branches. The experiment was repeated in two consecutive days, totaling 30 flowers in each treatment to allow observation and sampling of the two treatments simultaneously (Freitas and Alves, 2008).

2.7. Quantification of the number of visits of floral visitors in the experiments I and II

To test the hypothesis that the patrolling ants associated with extrafloral nectaries (EFNs) reduces the number of pollinator visits, the inflorescences were observed in the field for intervals of 10-min from 06:00 to 12:00 am.

The data collected was the number of pollinator visits to the plant, the total time each pollinator spent inside each flower, ant species patroling the plant, ant abundance in each flower, at the beginning and at end of the observation period. Two observers performed the quantification of the number of visits in each flower triad simultaneously. So that each one observed five triades, quantifying the number of visits on each trio of flowers for 10 minutes using the "observation window" where every window was 10 min, totaling 50 min of every hour (Freitas and Alves, 2008). We consider each landing in the flower and each indentation was considered one "flower reject" (when individuals evaluate and then avoid landing) (Polatto and Alves Junior, 2008). The observations occurred 6 am to 1 pm, so that, was given an interval of 10 minutes per hour to avoid the influence of the collector on site. The experiment I had 42 hours and the experiment II had 28 hours of observation.

2.8. Statistical analysis

The relationship between the number of floral parts preyed per inflorescence and insects found in each period was analyzed using Pearson's linear correlation test. Differences in the number of hits between florivory treatments and patrolling ants was tested through analysis of variance (ANOVA) randomized in blocks. Before the analysis, the changes were tested by the Shapiro-Wilk test and when required, the data were transformed into log for its standardization. Statistical analyzes were tested at 5% significance level, through free software R 3.1.1.

3. Results

3.1. Floral attributes

The flowers are axillary and are grouped in inflorescences summit type with a mean of 20.47 ± 14.24 budsbuds per inflorescence (n = 78 inflorescences) ranged from 1 to 59 budsbuds in different stages of development. The availability of flowers per inflorescence varied from 1 to 8 flower with a mean 2.23 ± 1.47 flowers per inflorescence. The flowers are exteriorized out of the foliage, actinomorphic, gamopetalous of campanulate type with straight tube widening gradually towards the apex, but allowing much of the corolla can be used as a landing pad. It is evident internally a darker color in the bottom of the tube, in the weld lines of the five petals and at central portions, giving triangular shape on the outside of floral tube, which act as nectar guides.

The corolla has an average 93.46 ± 5 mm in diameter. Floral tube has an average 30.88 ± 6 mm height and 9.42 ± 2 mm in diameter (n = 30), the color ranged from magenta-pink to purple.

3.2. Record of natural florivory

A total of 145 flowers and 1331 floral buds was analyzed in 78 inflorescences, registering an average of 2.23 ± 1.47 flowers per inflorescence and on mean 20.47 ± 14.24 buds per inflorescence. The total of flowers with some type of herbivory was 90.3% and buds totaled 7.3%.

The mean total number of flowers in the morning was 2.45 ± 1.7 and 16.02 ± 14.1 buds, the mean of damaged flowers was 1.7 ± 2.17 (86%; n = 86) and damaged buds was 1 ± 1.9 (6.8%; n = 561).

In the afternoon the mean total flowers per inflorescence was 1.9 ± 1.09 and the mean buds per inflorescence was 25.6 ± 12.7 , and the mean inflorescence flowers and buds

was predated by 1.9 \pm 1 (96.6%; n = 59) and 1.9 \pm 1.7 (7.7%; n = 770), respectively.

The total number of insects collected in inflorescences was 355, with 169 (47.60%) collected in the morning and 186 (52.39%) collected in the afternoon. Coleoptera individuals represented a total of 345 (97.18%), the others (2.81%) was represented by ants. Among the beetles, the most representative family was Nitidulidade (Cillaeinae) with 278 individuals (80.57%) followed by Chrysomelidae with 65 individuals (18.30%).

There was no significant positive relationship between damaged flowers (Morning: p = 0.2756; afternoon: p = 0.1702), damaged floral buds (Morning: p = 0.4820; afternoon: p = 0.476), damaged fruits (Morning: p = 0.5215; afternoon: p = 0.1369) and number of insects per inflorescence when calculated separately in both periods of the day.

But there was a significant correlation between the number of insects collected and floral parts damaged (flowers, buds and fruits), when counted together (Morning: r = 0.2715; GL = 74, p = 0.0176; afternoon: r = 0.2465; GL = 68; p = 0.0396).

3.3. Pollinators and ants visitants of EFNs

A total of 28 species of bees visited the flowers and 17 species of ants visited the extrafloral nectaries (as shown in Table 1 and 2). All bees acted as pollinators. The species Megachilinae sp.6 (87.5%), *Apis mellifera* Linnaeus,

 Table 1. Relative Frequency of pollinators in *Ipomoea carnea* subs. *fistulosa* (Convolvulaceae) collected (Aug of 2014 to Mar of 2015) in Tamanduá Farm, Santa Terezinha, Paraíba.

SUBFAMILIES	MORPHOSPECIES	FREQUENCY %	CATEGORY
Apinae	Apis melífera	75	Х
	Apinae sp	12.5	Z
	Xylocopa frontalis	75	Х
Halictinae	Augochlora sp.1	12.5	Z
	Habralictus sp.1	50	Y
	Habralictus sp.2	12.5	Z
	Habralictus sp.3	12.5	Z
	Oragapostemon sp.1	12.5	Z
	Paroxystoglossa sp.1	25	Y
	Paroxystoglossa sp.2	12.5	Z
	Pseudoaugochlora sp.	50	Y
Megachilinae	Megachile sp.1	25	Y
	Megachile sp.2	50	Y
	Megachile sp.2	12.5	Z
	Megachilinae sp.1	37.5	Y
	Megachilinae sp.2	37.5	Y
	Megachilinae sp.3	12.5	Z
	Megachilinae sp.4	25	Y
	Megachilinae sp.5	50	Y
	Megachilinae sp.6	87.5	Х
	Megachilinae sp.7	25	Y
	Megachilinae sp.8	37.5	Y
	Megachilinae sp.9	12.5	Z
	Megachilinae sp.10	12.5	Z
	Megachilinae sp.11	12.5	Z
Panurginae	Panurginae sp.1	37.5	Y
	Panurginae sp.2	37.5	Y
	Panurginae sp.3	25	Y

X = Constant; Y = Accessory; Z = Accidental.

1758 (75%) and *Xylocopa frontalis* Olivier, 1789 (75%) were classified as constants.

The Formicidae family was represented by three subfamilies: Formicinae, Myrmicinae and Pseudomyrmecinae. The species classified as constants belong to the subfamily Formicinae, among these are *Camponotus crassus* Mayr, 1862 (100%), *Dorymyrmex* sp.1 (75%) *Dorymyrmex* sp.2 (75%), *Camponotus* sp.3 (62.5%).

3.4. Florivory effects on the pollinators

A total of 1.194 visits were recorded bees, 1.141 of which occurred in the control flowers and 53 visits occurred in treatment with flowers mechanically damaged (see Figures 2 and 3). There was only one avoidant behavior of bee, which occurred in one flower of the treatment 2.

The simulated florivory (mechanical damage) affected the frequency of flower visitors, with a significant difference in the number of total visits between intact flowers and treatments (ANOVA randomized blocks: $F_{1.58} = 64.15$, p < 0.001; Tukey test ab = p < 0.001; c p = < 0.001; bc = p < 0.001) (see Figure 2).

3.5. Effect of patrolling ants associated with EFNs on pollinators

Only bees visited the flowers of *I. carnea* subs. *fistulosa* during the experiment. ants exhibited a very aggressive behavior during their visits to flowers, and on many occasion, the presence of ants seem to influenciate in the behavior of bees, that did not land in the flower. In other cases, visitors landed in bloom, but were soon chased away by ants. The total number of visits on flowers of branches with ants was 248, while in the branches without ants was 737.

Table 2. Frequency of visitors ants associated to the extrafloral nectaries *Ipomoea carnea* subs. *fistulosa* (Convolvulaceae) collected between August 2014 and March 2015 in Tamanduá Farm, Santa Terezinha, Paraíba.

SUBFAMILIES	MORPHOSPECIES	FREQUENCY
SUBFAMILIES	MORI HOSI ECIES	%
Formicinae	Brachymyrmex sp.1	25
	Camponotus crassus	100
	Camponotus sp.1	62.5
	Camponotus sp.2	50
	Camponotus sp.3	62.5
	Camponotus sp.4	50
	Camponotus sp.5	25
Dolichoderinae	Dorymyrmex sp.1	75
	Dorymyrmex sp.2	75
	Dorymyrmex sp.3	25
Myrmicinae	Cephalotes pusillus	37.5
	Crematogaster sp.1	37.5
	Crematogaster sp.2	12.5
	Solenopsis sp.1	75
	Solenopsis sp.2	12.5
	Solenopsis sp.3	12.5
Pseudomyrmecinae	Pseudomyrmex sp.1	37.5

There was a significant difference in the number of visits on flowers of branches with and without ants (ANOVA randomized blocks: $F_{1.58} = 106.5$, p <0.001) (see Figure 3), showing that the presence of ants in flowers

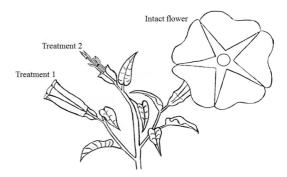
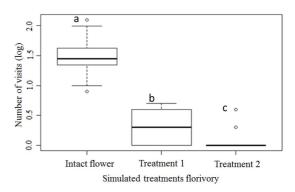
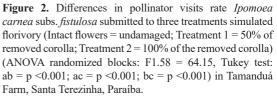


Figure 1. Schematic image of the simulated florivory treatments *Ipomoea carnea* subs. *fistulosa*: Treatment 1-50% of the corolla removed, treatment 2-100% of the corolla removed, Intact Flowers- undamaged.





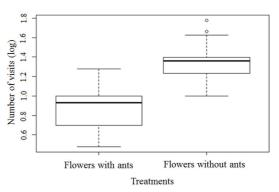


Figure 3. Differences in the number of pollinator visits in the absence and presence of ants in EFNs- Extrafloral nectaries (ANOVA randomized blocks: ($F_{1.58} = 106.5$, p <0.001); Tukey test p <0.001) in flowers of *Ipomoea carnea* subs. *fistulosa*.

of *Ipomoea carnea* subs. *fistulosa* affects negatively the occurrence of flower pollinator. In addition, there were 54 avoidant behavior.

4. Discussion

4.1. Floral attributes

The opening small number of flowers per inflorescence throughout the day is observed for other convolvulaceae of the Caatinga (Piedade, 1998), as well as for convolvulaceae of other vegetation types, as *Merremia dissecta* and *Merremia cissoides* (Maimoni-Rodella and Rodella, 1986) *Ipomoea hederifolia*, *I. quamoclit* (Machado and Sazima, 1987), *Ipomoea aristolochiaefolia* (Maimoni-Rodella, 1991), *Ipomoea acuminata* (Maimoni-Rodella and Rodella, 1992).

The exteriorisation of flowers outside the foliage confers a common exposure strategy between the Convolvulacea, being reported for other species of the family (Maimoni-Rodella and Rodella, 1986; Machado and Sazima, 1987; Piedade, 1998).

This exteriorisation increase the attractiveness for visitors. Additionally, features such as the presence of landing platform, nectary partially hidden, corolla with attractive colors, presence of nectar guides and diurnal anthesis are indicators of an evolutionary strategy developed to guarantee the success of the interaction with the group to which *I. carnea* subs. *fistulosa* is dependent for its pollination (Martins and Batalha, 2006). These characteristics are registered for other species of *Ipomoea* as *I. bahiensis* (Pacheco-Filho, 2010) and *I. asarifolia* (Kiill and Ranga, 2003).

4.2. Record of natural florivory

The small percentage of predated fruits can be explained according to optimal defense theory (Zangeri and Bazzaz, 1992). This is why not all parts of the plant have the same value for fitness. Therefore, more pieces that are valuable should contain more defenses. Therefore, fruits and seeds, in particular, tend to be very well protected.

The insects of the family Nitidulidae present in the flowers of *Ipomoea carnea* subs. *fistulosa*, are cited in papers such as some of the main pollinators (Silberbauer-Gottsberger and Gottsberger, 1988). However, in *I. carnea* subs. *fistulosa* the species of the genus *Conotelus* (Nitidulidae) consumed large amount of pollen grains even reaching to damage the anthers and stigma.

Species of the family Nitidulidae were responsible for damage in flowers of *Amphilophium vauthieri* (Bignoniaceae), in a semi-deciduous forest of Campinas (SP), damaging the fruiting of these lianas (Amaral, 1992). For many other species, the Nitidulidae family may consume fruits, seeds and pollen (Krupnick et al., 1999). However, the presence of the family in *Ipomoea carnea* subs. *fistulosa* flowers has not yet been recorded. For the Chrysomelidae family there are a large number of works directly related to species of the genus *Ipomoea* (Keeler, 1975; Crawley, 1983; Devall and Thien, 1989; Frey, 1995; Leavitt and Robertson, 2006.). In the present study, Chrysomelidae family was represented mainly by the species *Diabotrica speciosa* (Galerucinae). Which according to Haji (1981) and Gassen (1989) is a polyphagous pest that affects several crops in Brazil, in this way detailed studies on the role of these insects to the populations of *I. carnea* subs. *fistulosa* can be potential sources of biological control and should be performed in the future.

4.3. Pollinators and ants visitants of EFNs

The data obtained indicated that *Ipomoea carnea* subs. *fistulosa* is a mellitophylous species of promiscuous pollination, since several species of bees participate of the pollination process (see Table 1) and have easy access to the floral resources (Faegri and Van Der Pijl, 1980). *Ipomoea carnea* subs. *fistulosa* has flowering throughout the year and therefore, can be considered an important source of nectar for medium and small bees, such as the species that belong to the subfamilies Apinae, Halictinae, Megachilinae.

The species Megachilinae sp.6, *Apis mellifera* and *Xylocopa frontalis* were classified as constants. Pollination of other Convolvulaceae species by Megachilinae and Apis melifera was also recorded (Kiill and Ranga, 2003; Kiill and Simão-Bianchini, 2011).

Until then, the only article with Xylocopa frontalis visiting flowers of Ipomoea carnea subs. fistulosa was that of Keeler (1977), but citing them as nectar thieves and not properly pollinators, thus more detailed studies about the role of *Xylocopa frontalis* for pollination of *I. carnea* subs. *fistulosa* need to be carried out. Recent studies have shown that the abundance and diversity of ants-plant associations are particularly significant in the tropical region (Kokolo et al., 2016; Nascimento and Barbosa, 2018).

Similarly to *I. carnea* subs. *fistulosa*, the extrafloral nectary are present in other representatives of the genus *Ipomea* L. (Keeler, 1975, 1977; Mondal et al., 2013), the most representative genus of the Convolvulaceae family.

Ants of the genus *Camponotus*, *Dormyrmex* and *Pseudomyrmex* (see Table 2), are frequent visitors of nectariferous glands in Convolvulaceae, since in most studies such genera of Formicidae are mentioned (Keeler, 1975; Mondal et al., 2013; Paz et al., 2016). The frequency of ants of genera citeded was observed during the study period, which may suggest that this an important source of food resource for these ants present in semiarid environments.

4.4. Florivory effects on the pollinators

The results of the effect of floral damage (e.g. partial or total loss of the corolla) showed that florivory decreases the number of total visits. Observing the behavior of visitors bees, we identified a high degree of recognition both of floral traits as the risk of predation, which influenced the outcome of the visitation rates. Insects of this order are known for their high ability to recognize visual cues, such as shape, symmetry, color and also olfactory signals of flowers that they visit (Wignall et al., 2006). The smallest number of pollinator visits in damaged flowers can be justified by the fact that the floral damage in *I. carnea* subs. *fistulosa* consisted of the loss of floral attributes important. Characteristic of the genus *Ipomoea*, as corolla with attractive colors, nectar guides and landing platform, which are attractiveness components for pollinators, for they indicate availability of floral resources of high quality (Paz and Pigozzo, 2012).

The floral damage many times caused by florivoros, besides reducing the pollination activity of insects floral visitors (McCall, 2008), may affect other behaviors of these pollinators, such as the preference of some of these insects laying eggs on flowers without damage to the stigma (Horn and Holland, 2010).

The influence of floral damage on the avoidance behavior of insects, generally could be explained by the lesser quality of flowers plant in whom occurred these effects (Strauss, 1997; Lehtila and Strauss, 1999; Mothershead and Marquis, 2000; Narbona and Dirzo, 2010) causing the flowers become less attractive and more avoided this way.

4.5. Effect of patrolling ants associated with EFNs on pollinators

Although EFN ants have a defensive function against herbivory as described by Frey (1995), the species *I. carnea* subs. *fistulosa* presented high rates of florivoria in the study area, and it is necessary to carry out more studies that focus on the cost / benefit relation of the association between plants and ants. Because for this study system, although the presence of ants consisted of a biotic defense strategy against herbivory, ant-plant interactions affected the plant-pollinator system.

A possible explanation for the reduction of bee visits in the presence of a potential predator, is that bees can evaluate the pattern of flower symmetry to the distance (Leonard et al., 2011) and detect predators (Abbott, 2010; Defrize et al., 2010).

With the approaching of the bee to the flower, the ants can be efficiently detected (Defrize et al., 2010) and the bee can give up to complete the visit. After choosing the flower, the visitor has spent time and energy in search of his resource. Therefore, avoidance behaviors flowers before accessing the nectaries occurs in response to the perceived predator for bees, which corroborates the predator-prey recognition system (Dukas, 2001; Suttle, 2003).

Interactions between ants and plants are well documented, in relation to the benefits offered by this interaction, however the consequence multitrophic interactions can result in greater or lesser plant reproductive successbecause ants foraging in EFNs attack and drive away the floral visitors, whether or not pollinators.

The flowers in the presence of predators (ants) influenced the avoidance behavior of the bees, unlike floral damage, in which only an avoidance behavior was registered. Possibly, this increased behavioral response in avoiding a potential predator occurs due to the strong pressure exerted by predation. It should be noted that the ability to detect and avoid these risks allows the visiting insects of flowers to reduce the probability of being captured, thus increasing the aptitude of the pollinators as a whole (Abbott and Dukas, 2009; Ings and Chittka, 2009).

On the other hand, the reduction of the quality of floral resources caused by herbivory (Krupnick et al., 1999) may not have an evolutionarily strong effect on predator-prey interactions.

The close relationship between predator, plant and their pollinators presented here, corroborates the hypothesis that predators such as ants chase pollinators from plant pollination systems (Romero and Koricheva, 2011).

These effects on *Ipomoea carnea* subs. *fistulosa* may be considered risky, since it is a self-incompatible species (Martin, 1970; Proctor et al., 1996) and depends on the activity of the bees for your fertilization (Schlising, 1970; Kiill and Ranga, 2003). The same applies the species with this fertilization system.

However, further studies that include other ecosystems, other species of predators, plants and floral visitors are needed to broaden the understanding of indirect interactions, and mainly to understand the factors that lead to variations in the results of these relationships.

Acknowledgements

This work was carried out with financial support from by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – PQ-307422/2012-7). The first author had a CAPES MSc./Masters scholarship; Field logistic support was given by Instituto Tamanduá and Mr Pierre Landolt, owner of Fazenda Tamanduá.

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