Psyche

Plant-Arthropod Interactions: A Behavioral Approach

Guest Editors: Kleber Del-Claro, Monique Johnson,
and Helena Maura Torezan-Silingardi

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Editorial

Plant-Arthropod Interactions: A Behavioral Approach

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In a community, the species present may be linked directly or indirectly through resources and consumption. More than 90% of the fixed energy in terrestrial systems is processed autotrophically by plants and thus almost all terrestrial fauna depend on this production. Classically, in systems involving three trophic levels, such as plants, herbivores, and predators, a trophic cascade describes the positive top-down effects of the third trophic level on the biomass, richness, or composition of the producer species (see [1] and references therein).

In a more recent and realistic approach, biodiversity is viewed and evaluated in ways that embrace the extreme richness inherent in plant-animal interactions, including not only trophic relationships, but also aspects of life histories, biology, and behavior of interacting species [1]. The richness of biotic interactions has been identified as the main force responsible for the biodiversity that maintains viable communities. Indeed, the success of life on Earth is directly linked to the success of biotic interactions [2]. Biotic interactions are present everywhere, in air, water, soil, and on or inside organisms regardless of their size.

Among all of these interactions, those between plants and animals have been described as being largely responsible for the functioning and maintenance of trophic chains in ecosystems and also for the patterns and processes that structure biodiversity on Earth [3]. This idea was born of the hundreds of papers published in the last century, mainly between 1960 and 1990, involving mutualistic and antagonistic plant-animal relationships (Figure 1). In these studies, the outcomes of interactions were almost always related to aspects of the natural history of the interacting plant and/or animal species involved (e.g., [4, 5]). In addition, many of these studies revealed that the final interaction results often differed as a function of season and/or year, thus clearly demonstrating the importance of studying the natural history of interacting species in order to truly further our understanding of the existing interactions [6]. Also, the outcomes of interactions may vary as a function of the physical and biotic changes in the environment. Thus, there is no mutualistic relationship that will be always and unconditionally one of mutualism. Similarly, predatory or parasitic relationships may be subject to changes occurring more or less quickly over evolutionary time that can drive the results of the interaction in a different direction.

Although greatly recognized as relevant, basic knowledge concerning species interactions, including plant phenological variations, features of life histories, and animal behavior, is surprisingly rarely considered in the more recent studies of plant-animal network interactions [7]. In this special volume, we have selected papers in order to draw attention to these interaction network studies as they are fundamental to the comprehension of specialization patterns in plants and animals [8]. We have decided to center our attention on arthropod-plant relationships in this volume, mainly due to the entomological scope of the journal but also due to the fact that arthropod-plant interactions exhibit a wide variety of relationships. Readers will thus discover here a myriad of interaction possibilities in the different articles, from more general and theoretical studies to basic and natural history studies in arthropod-plant interactions. We hope that this special volume will both exemplify how these interactions

(a) (b)

Figure 1: The Malpighiaceae (*Peixotoa tomentosa*) has its flowers visited by (a) the pollinator bee (*Centris* (*Ptilotopus*), Apidae) and (b) also (like in Banisteriopsis laevifolia) by the small black thrips (*Heterothrips peixotoa*, Heterothripidae) that infest and destroy its stamens and stigma.

can contribute to the general ecological theory and reinforce the value of natural history and behavioral studies.

Kleber Del-Claro
Monique Johnson
Helena Maura Torezan-Silingardi

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Research Article

Might Heterostyly Underlie Spider Occurrence on Inflorescences? A Case Study of *Palicourea rigida* (Rubiaceae), a Common Shrub from Brazilian Cerrado

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We carried out a research on the *Palicourea rigida* (Rubiaceae) inflorescences, a distylous shrub of Brazilian Cerrado. Our objective was to compare the inflorescence architectural complexity and its quality in the two floral morphs and search for any relationship with spider occurrence. In order to assess the quality of inflorescence resources, we quantified the nectar volume and its sugar concentration and the number of fruits and flowers (intact and aborted) for both inflorescence morphs with and without spiders. For the architectural heterogeneity, we quantified floral structures and inflorescence levels of branching. Spider occurrence was higher in longistylous inflorescences than in brevistylous ones. The sampled spiders were classified into the guilds ambushers, jumpers, or orb-weavers. Ambushers, jumpers, and total richness were much higher among longistylous inflorescences. We found no difference between morphs neither in volume or nectar concentration nor in amount of fruits and flowers. However, longistylous inflorescences presented greater architectural heterogeneity than brevistylous ones. Therefore, we suggested that architectural heterogeneity is an important factor underlying the occurrence of cursorial spiders on *P. rigida* inflorescences, which possibly arose from the relationship between refuge availability and inflorescence architecture.

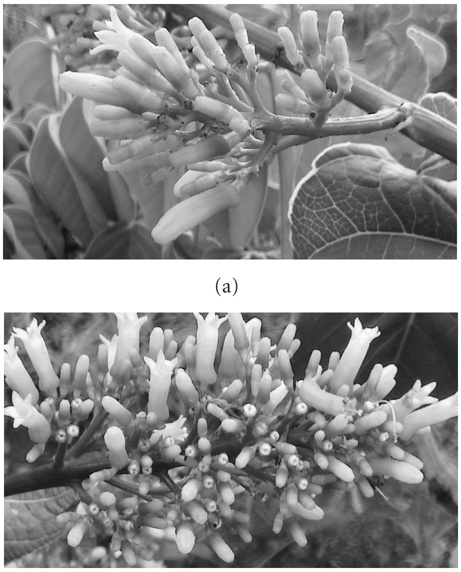
1. Introduction

Spiders occur in almost all land environments, but more abundantly in vegetation-rich areas [1]. They frequently use plants as substrate to forage and represent one of the main predators that control phytophagous arthropods in natural and agricultural systems [2, 3]. Furthermore, spiders also represent a key-assemblage to plants, since they play a potentially critical role over the dynamics of local trophic networks [4, 5]. Consequently, basic knowledge of what regulates spider richness and abundance is crucial to understand the dynamics of the arthropod community on plants.

Spiders can select substrate based largely on cues from host plant architecture, which may influence the spider assemblage distribution [4, 6, 7]. It has been shown, for example, that spider richness and abundance may be influenced by the density of branches [8], leaves [7], and spines [4] by the leaf morphology [9] and by the availability of plant structures distributed vertically [10, 11]. Although the patterns between spider diversity and plant architectural complexity have been frequently demonstrated (e.g., [4, 6, 7, 12]), the causal mechanism which determines these patterns has yet to be identified, and different physical [13] and biotical [5] components of the habitat should be involved. Among the possible causal mechanisms pointed out by the literature are refuge against intraguild predation, increases of prey abundance, refuge against physical disturbances, and providing of milder microclimatic conditions [13].

Inflorescences are more propitious to colonization by spiders once they present high prey abundance, favourable microclimate conditions, and refuge availability against predation [14, 15]. However, the habitat patch choice by spiders can be influenced not only by the reproductive branch architecture, but also by the flower or inflorescence quality, such as nectar production and flower senescence state [16]. Since some flowers could be more or less attractive to spiders establishment and, therefore, present a more or less diverse spider assemblage, it would be possible that this differential spider distribution leads to variations of phytophagous impact on floral structures or seeds and, consequently, in reproductive success. For instance, [3] it was experimentally demonstrated that the spiders on or at least near inflorescences can influence herbivory rate, as well as visits by pollinators, which, by the way, leads to straight consequences for the plant adaptive value.

The species *Palicourea rigida* H.B.K. (Rubiaceae) is a common shrub (0.5–1.5 m tall) from Cerrado, a Brazilian savannah-like system, with a simple vegetative structure (1-2 stems and few large leaves). However, this plant species presents a very variable architecture regarding its reproductive branches, since inflorescences can have 1 up to 6 branching orders as well as from few up to hundreds of flowers. Even after the fall of the corolla, the nectaries still remain active in plants, as for other Rubiaceae species [17]. The plant of the *P. rigida* can be allocated to two distinct morphs (i.e., distyly): brevistylous or longistylous (Figure 1). The heterostyly in *P. rigida* may be distinguished through inflorescence architectural complexity and quality. So, the objectives of this study were (1) to compare the architectural complexity and spider occurrence between the two morphs of *P. rigida*; (2) to compare the inflorescence quality, which in this study we took as the nectar volume and sugar concentration, and as the number of fruits and flowers, intact and aborted, in the two morphs of *P. rigida* and in the presence and absence of spiders. Therefore, we used the *P. rigida* shrubs as a case study to test the following hypotheses: (1) spider richness would be higher on the morph with the highest architectural complexity; (2) the habitat (i.e., inflorescence) quality differ between brevistylous and longistylous morphs; (3) higher habitat quality, such as greater volume and sugar concentration, larger numbers of fruits and intact flowers, and lower number of aborted flowers, is positively correlated to the presence of spiders on brevistylous and longistylous inflorescences.


(a)

(b)

Figure 1: The *P. rigida* inflorescences: (a) brevistylous morph and (b) longistylous morph. Note the difference between the two morphs with regard to the architectural complexity.

2. Materials and Methods

2.1. Area of Study. The study was conducted in Caldas Novas Mountain State Park (PESCAN), in south-eastern of Goiás state, at Brazil midwest (17°43′56′′ S to 17°50′55.7′′ S and 48°40′0′′ W to 48°42′57.6′′ W). The Park has 12,500 ha, where Cerrado, tropical savanna-like vegetation, is predominant. The climate in the region has two well-defined seasons: dry from May to September and wet throughout the months of November, December, and January [18].

2.2. Studied Plant Species. The shrub *Palicourea rigida* H.B.K. (Rubiaceae) has its flowering season from September to March and fructifies from November to April. Additionally, *P. rigida* is the most common *Palicourea* species of the Brazilian Cerrado [19–21]. This species presents heterostyly of the distylous type (i.e., brevistylous and longistylous) (Figure 1), with the size and shape of stigmas and anthers differing between both of morphological types [22]. Heterostylous plants usually possess a self-incompatibility system, where fruit set occurs only after cross-pollination between morphs (legitimate cross-pollination) [21]. The species is pollinated by hummingbirds [22].

2.3. Spider Sampling. In the middle of the flowering season of 2008 in an area of 15,000 m^2, we marked all the 36 shrubs of *P. rigida* with reproductive branches and with open flowers at a transect of 200 m long and 75 m wide using a global positioning device. As for each individual dealt with, we inspected, classified, and labelled the reproductive branches according to morphs of flowered inflorescence. Summing up, we observed whether they belonged to brevistylous or longistylous types. From the 36 plants, 15 were brevistylous and 21 were longistylous. We randomly selected one inflorescence from each of these 36 shrubs and collected manually the spiders on them. The spiders sampled were identified to the species level or assigned to a morphospecies. We also assigned the different spiders species or morphospecies sampled on *P. rigida* inflorescences to three different guilds: (1) ambushers, which included spiders thatdo not build snare webs for capturing prey and forage by sit-and-wait strategy—in this study, this guild was only composed by the family Thomisidae; (2) jumpers, which comprised spiders that do not build snare webs for capturing prey and hunt actively, often by jumping on their prey—the guild was composed by the families Salticidae and Oxyopidae; (3) orb-weavers which included spiders that build snare orb-webs for capturing prey—the guild was composed only by the family Araneidae. In this study, we referred to the nonweb-building guilds of jumpers and ambushers as cursorial spiders.

2.4. Architectural Heterogeneity. Firstly, we measured the architectural heterogeneity through quantifying the number of primary, secondary, tertiary, quaternary, quinary, and senary branches, if they occurred. also, we quantified the number of different structures (such as closed and open buds, floral abortions, and infrutescences) presented in the distal part of each inflorescence and measured inflorescence length. For these measures, we sampled two inflorescences from eight shrubs of each morph. Finally, we divided, for each inflorescence sampled, the branch quantity from each branching level and the number of structures by their respective inflorescence length standardization of the architectural heterogeneity measures for inflorescences of different sizes. Furthermore, we also took note about spider presence or absence on the sampled inflorescences.

2.5. Sugar Concentration and Nectar Volume Produced. We used glass graduated micropipettes (20 μL) for extracting and measuring the volume of nectar, whereas for measuring sugar concentration, we used the handheld refractometer ITREF-90. For nectar and sugar measures, we sampled flowers from six randomly chosen brevistylous plants and five longistylous ones. From each plant, we sampled randomly two inflorescence and used, according to the availability of open flowers, 4.42 ± 0.38 (mean ± SE) flowers per inflorescence for brevistylous plants and 4.3 ± 0.47 for longistylous ones. We bagged the inflorescences with organza one day before the measurement in order to avoid contact with floral visitants. As nectar is produced continuously throughout the day, the volume and concentration measures were standardised for the same period of the day, between nine and eleven o'clock in the morning. We also took note whether the sampled inflorescence had spider on it or not.

2.6. Abundance of Fruits and Flowers. In plants where spiders occurred in some of their inflorescences, we randomly selected, from the same shrub, two inflorescence, with similar size and architecture, but one with spiders and another without. In both of these inflorescences, we quantified the number of intact and aborted flowers as well as the growing fruit. A total of 5 brevistylous and 15 longistylous plants were utilized. This difference in sampling size between the two morphs was unavoidable, since brevistylous inflorescences with spiders associated were far more scarce than longistylous ones.

2.7. Data Analysis. In order to compare the spider guild richness per inflorescence and the proportion of shrubs with spiders between brevistylous and longistylous morphs, we run the nonparametric bilateral Mann-Whitney U-test and χ^2 test, respectively. The number of spider species associated with the two *P. rigida* morphs was estimated by Coleman rarefaction curve. We tested whether the nectar volume and sugar concentration differed between the two morphs and between inflorescences with and without spiders. Once we collected two inflorescences from each *P. rigida* individual, the nested ANOVA with type III sum of squares was chosen for comparisons, since the inflorescence factor was nested within individual factor, which, in turn, was nested within morph factor or spider presence/absence factor. We considered inflorescence as individual random factors, whereas the morph or the spider presence/absence we considered as fixed factors. In order to compare the amount of fruits, intact and aborted flowers between inflorescence with and without spiders, we performed paired t-tests for each morph. We also tested, by performing bilateral Mann-Whitney U-tests, whether the amount of fruits and flowers differed between brevistylous and longistylous inflorescences, with spiders and without spiders. We compared the architectural heterogeneity between brevistylous and longistylous inflorescences through the principal component analysis (PCA) of correlation with a centered matrix, once the data set included eight architectural variables. The PCA matrix was set up by putting each sampled inflorescence as objects and architectural variables as the object attributes. By performing PCA, we could visualize general architectural patterns for both morphs as well as how the different architectural variables covariate with each other, allowing us to detect what architectural variables are most likely responsible for the differentiation between brevistylous and longistylous morphs. We used the software Statistica 7.0 (StatSoft) to perform the Mann-Whitney U-Test, χ^2 test, nested ANOVA, and paired t-test, whereas for multivariate analysis of PCA and for estimation of species number by Coleman rarefaction, we used the softwares FITOPAC 2.1.2.85 [23] and EstimateS 7.5.0 [24], respectively. For the nested ANOVA and paired t-test, we transformed the response variable data set into log $(n + 1)$ in order to normalize and equalize the variances for parametric tests. We assumed the level of statistical significance as $P < 0.05$ for all analyses performed.

3. Results

3.1. Spider Occurrence and Richness. Among the inflorescences with spiders on them, just about one or two spiders were collected per inflorescence. So, the spider richness on *P. rigida* inflorescence was a measure close to the spider abundance. Although the abundance of spiders per inflorescence was low, the spider presence/absence was still remarkably discrepant between the two floral morphs. The proportion of shrubs with adult spiders differed between

Table 1: Frequency of spider morphospecies sampled on longistylous and brevistylous inflorescences of *P. rigida*.

Spider species and morphospecies	Frequency on longistylous inflorescences (%)	Frequency on brevistylous inflorescences (%)
Oxyopidae		
Oxyopes salticus Hentz, 1845	23.81	—
Thomisidae		
Misumenops sp.1	19.04	—
Deltoclita sp.1	9.52	—
Deltoclita sp.2	4.77	—
Salticidae		
Corythalia sp.1	38.09	13.3
Araneidae		
Araneus sp.1	4.77	—
Total	21	2

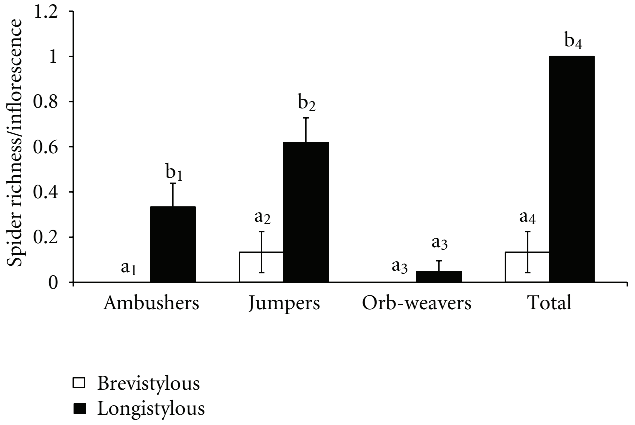


Figure 2: Spider guild richness per inflorescence in brevistylous and longistylous shrubs of *P. rigida*. Error bars represent ± SE. The unequal letters above error bars indicate statistically significant differences at significance level of 0.05.

brevistylous and longistylous morphs ($\chi^2 = 28.49$, df = 1, $P < 0.0001$), since 2 out of 15 brevistylous shrubs sampled featured spiders on their inflorescences, whereas all the longistylous ones had spiders. We have found a total of six different spider species belonging to four families on *P. rigida* inflorescences (Table 1). Cursorial spider guilds were predominant on *P. rigida* inflorescences, since *Corythalia* sp.1 (Salticidae) was the most frequent species (38.09%), followed by *Oxyopes salticus* Hentz, 1845 (Oxyopidae) (23.81%), and *Misumenops* sp.1 (Thomisidae) (19.4%) (Table 1). Only *Corythalia* had often been found inside silk structures, which were frequently built on inflorescence extremities and used as retreats. The retreat of each spider individual occupied about one third of the inflorescence length.

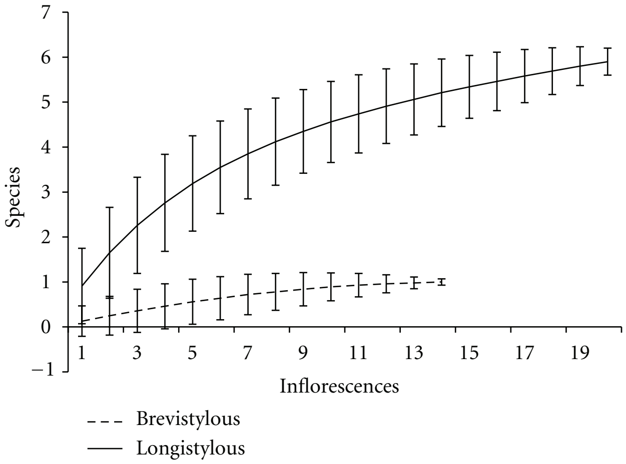


Figure 3: Coleman rarefaction curve for samples of brevistylous and longistylous inflorescences. Each inflorescence sample represents one distinct individual of *P. rigida*. Error bars across the two curves represent ± SD.

Total spider richness per inflorescence was higher among longistylous shrubs than among brevistylous ones (Mann-Whitney U-test; $z = -5.263$, $P < 0.001$) (Figure 2). The same happened to the spiders of the guilds of Ambushers (Mann-Whitney U-test; $z = -2.456$, $P = 0.014$) and Jumpers (Mann-Whitney U-test; $z = -2.873$, $P = 0.004$) (Figure 2). However, the richness per inflorescence of the Orb-weavers guild did not differ between brevistylous and longistylous morphs (Mann-Whitney U-test; $z = -0.845$, $P = 0.398$). The estimated total number of spider species by the Coleman rarefaction method was higher for longistylous inflorescences, since the estimate was about five up to six species for longistylous and only one species for brevistylous (Figure 3). Thus, although each *P. rigida* inflorescence with spider on it presented just about one spider species, all the longistylous inflorescences sampled sheltered about five more spiders than the brevistylous ones.

3.2. Architectural Heterogeneity. The PCA performed for the architectural variables of inflorescences explained about 60% of all proved successful [25] (Table 2). On the plane formed by the first two PCA axes, we can observe the general tendency of longistylous inflorescences being architecturally more complex than brevistylous ones (Figures 1 and 4). Longistylous inflorescences tend to have higher density of different floral structures, such as closed or open buds, aborted flowers, and infrutescences. The density of higher order branches (such as secondary, tertiary, quaternary, and quinary branching) appears to be positively correlated to each other and more abundant among longistylous inflorescences. Although there is overlapping regarding architecture features between the two morphs, which is expected since the morphs belong to the same plant species, longistylous inflorescences seem to reach more extreme values among most architectural traits, which was responsible for much of the variance embraced in the first PCA axis (Figure 4).

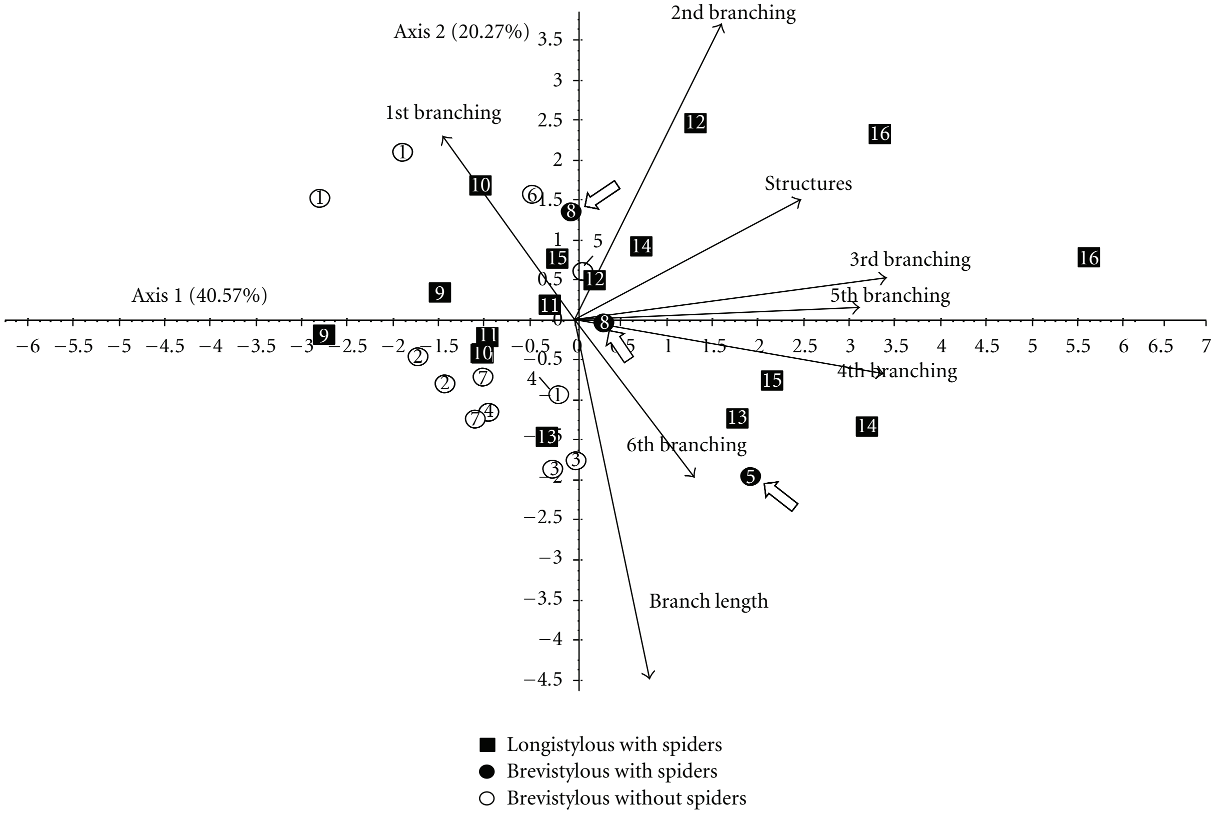


Figure 4: Graphic representation for the first two axes of the PCA performed with the architectural variables of *P. rigida* inflorescences. Objects: sampled brevistylous and longistylous inflorescences, the object number indicates the number of the sampled *P. rigida* shrub. Vectors and Structures: number of floral structures such as open and closed buds, floral abortions, and infrutescences; branch length: total inflorescence length; 1st, 2nd, 3rd, 4th, 5th, and 6th branching: density of primary, secondary, tertiary, quaternary, quinary, and senary branches, respectively.

The pattern of spider presence/absence on inflorescences also seems to follow the pattern of architectural complexity, since spiders tend to occur on architecturally more complex inflorescences. It is also noteworthy that the few brevistylous inflorescences with associated spiders present architectural features relatively more complex than the other brevistylous, such as greater density of secondary and senary branches (Figure 4, block arrows).

3.3. Sugar Concentration and Nectar Volume Produced. There was no difference neither in nectar volume ($F_{1,74} = 0.243$, $P = 0.633$) nor sugar concentration ($F_{1,74} = 0.855$, $P = 0.378$) between the two morphs (Figure 5). There was also no difference between inflorescences with and without spiders regarding to nectar volume ($F_{1,74} = 0.538$, $P = 0.48$) or sugar concentration ($F_{1,74} = 0.35$, $P = 0.566$ (Figure 5). Indeed, nectar volume and sugar concentration varied significantly from individual to individual, regardless of which morph it belonged to (nectar volume: $F_{9,74} = 4.828$, $P = 0.008$; sugar concentration: $F_{9,74} = 14.8$, $P < 0.0001$) or of the spider presence (nectar volume: $F_{9,74} = 7.721$, $P = 0.001$; sugar concentration: $F_{9,74} = 4.045$, $P = 0.014$). So, we do not have evidence for difference in nectar volume or sugar concentration between the two morphs or between inflorescences with and without spiders.

Table 2: Eigenvalues, cumulative percent variation, and eigenvectors of the first three principal components axes for the eight architecture variables of longistylous and brevistylous *P. rigida* inflorescences.

	Axis 1	Axis 2	Axis 3
Eigenvalue	3.246	1.622	0.883
Percent variation	40.57	20.27	11.04
Percent variation as expected by the broken stick model	33.97	21.47	15.22
Eigenvectors			
1st branching	−0.2123	0.3369	−0.4324
2nd branching	0.2382	0.5445	0.2173
3rd branching	0.5034	0.0770	0.0039
4th branching	0.4984	−0.1012	−0.1052
5th branching	0.4591	0.0212	−0.4433
6th branching	0.1925	−0.2925	0.6737
Branch length	0.1218	−0.6626	−0.3186
Structures	0.3658	0.2208	0.0536

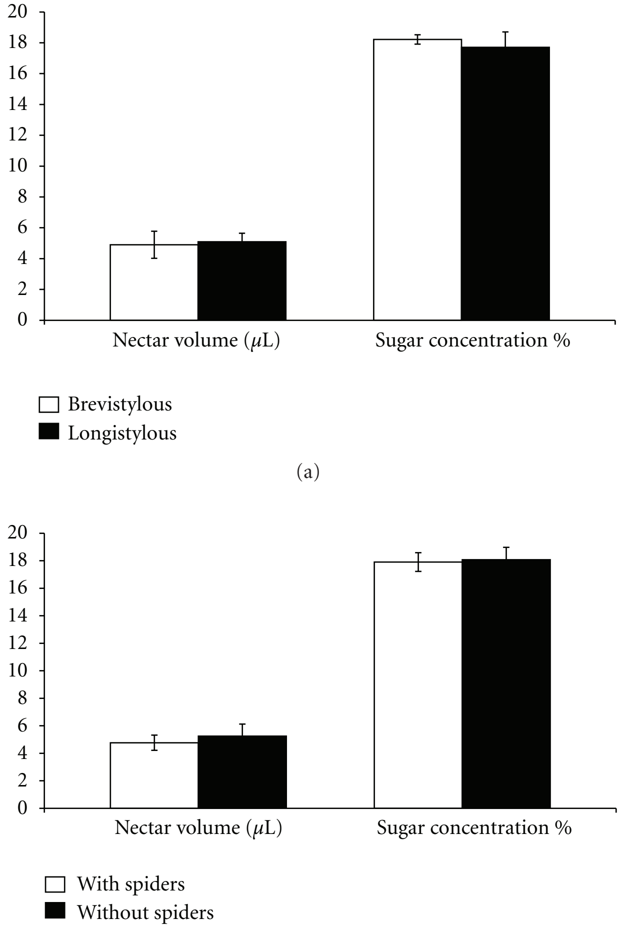


FIGURE 5: Nectar volume and sugar concentration between (a) brevistylous and longistylous morphs and between inflorescences (b) with and without spiders. Error bars represent ± SE.

3.4. Abundance of Fruits and Flowers. The differences in the amount of fruits, intact flowers, and aborted flowers between inflorescences with and without spiders were not significant for both brevistylous (paired t-test fruits: $t = 0.241$, df = 4, $P = 0.821$; intact flowers: $t = -1.362$, df = 4, $P = 0.245$; aborted flowers: $t = -2.234$, df = 4, $P = 0.089$) and longistylous morph (paired t-test fruits: $t = 1.685$, df = 12, $P = 0.118$; intact flowers: $t = -0.054$, df = 12, $P = 0.958$; aborted flowers: $t = 0.343$, df = 12, $P = 0.737$) (Figure 6). There was also no difference in the amount of fruits and flowers (intact or aborted) between brevistylous and longistylous inflorescences with spiders (Mann-Whitney U-test fruits: $z = 1.178$, $P = 0.239$; intact flowers: $z = 1.004$, $P = 0.315$; aborted flowers: $z = 0.829, P = 0.407$) and without spiders (Mann-Whitney U-test fruits: $z = 0.000$, $P = 1.000$; intact flowers: $z = 1.626$, $P = 0.104$; aborted flowers: $z = 1.128, P = 0.200$). Therefore, we did not find any evidence for a relationship between the inflorescence morph or the spider occurrence and the abundance of fruits and flowers (intact or aborted) in *P. rigida* inflorescences.

4. Discussion

The spider occurrence pattern was remarkably distinct between the two morphs, with most of the spiders occurring on the longistylous morph. The total spider richness was also higher on longistylous inflorescences than on brevistylous ones. Thus, the evidence supports the view that there is some sort of difference in habitat quality between these two morphs. However, the two morphs differed more consistently only with respect to architectural heterogeneity, since longistylous inflorescence is, on average, architecturally more complex than brevistylous. Therefore, this study presents some evidence in favor of the structural heterogeneity hypothesis [26, 27] regarding the spider occurrence pattern on related inflorescences. The habitat architecture actually is often related to the availability of refuge for protection against predators and abiotic adversities, such as physical disturbance and harsh climatic conditions [13]. The higher refuge availability against intraguild predation can result in a higher predator richness and abundance in architecturally complex environments [28, 29]. Furthermore, complex architecture provides more attaching points for spider silk structures, such as snare webs and retreats [4, 15]. So, the higher spider occurrence and total richness on longistylous inflorescences may then be explained, at least partially, by their greater architectural complexity due to increasing shelter availability and suitability for building silk structures.

We have no evidence that other characteristics related to habitat quality in *P. rigida* inflorescences, such as nectar volume, sugar concentration, and quantity of flowers and fruits, are in some way correlated to the presence of spiders. Such characteristics are usually related to the prey abundance on inflorescences, since they are largely responsible for attracting pollinators and phytophagous [30–32]. However, we should note that *P. rigida*, like many other of Rubiaceae family [33], is an ornithophilous species that is primarily pollinated by hummingbirds, with insects such as bees, moths, and flies only occurring as occasional visitors [21]. For example, [14] compared inflorescences of an ornithophilous species (*Palicourea guianensis*, Rubiaceae) with a species pollinated by bees and butterflies (*Lantana camara*, Verbenaceae) and found that the ornithophilous one had much lower density of insects and spiders than the one pollinated by insects. Since insect pollinators represent an important prey source for spiders on inflorescences [34, 35], it is possible that, for ornithophilous species such as *P. rigida*, prey availability would not be a main factor influencing foraging substrate choice by spiders on inflorescences, rather other factors would be more important such as refuge availability provided by the complex architecture of reproductive branches. In [6], for example, even artificial inflorescences, which were very poor in prey, attracted more spiders than vegetative branches, indicating that spiders can select habitat based only on architecture *per se*. Taking into consideration the significant importance of tactile signals for the environment perception by spiders [1, 35], it is possible that the architectural configuration of plant structures is one of the main cues used by spiders in selection of foraging substrates.

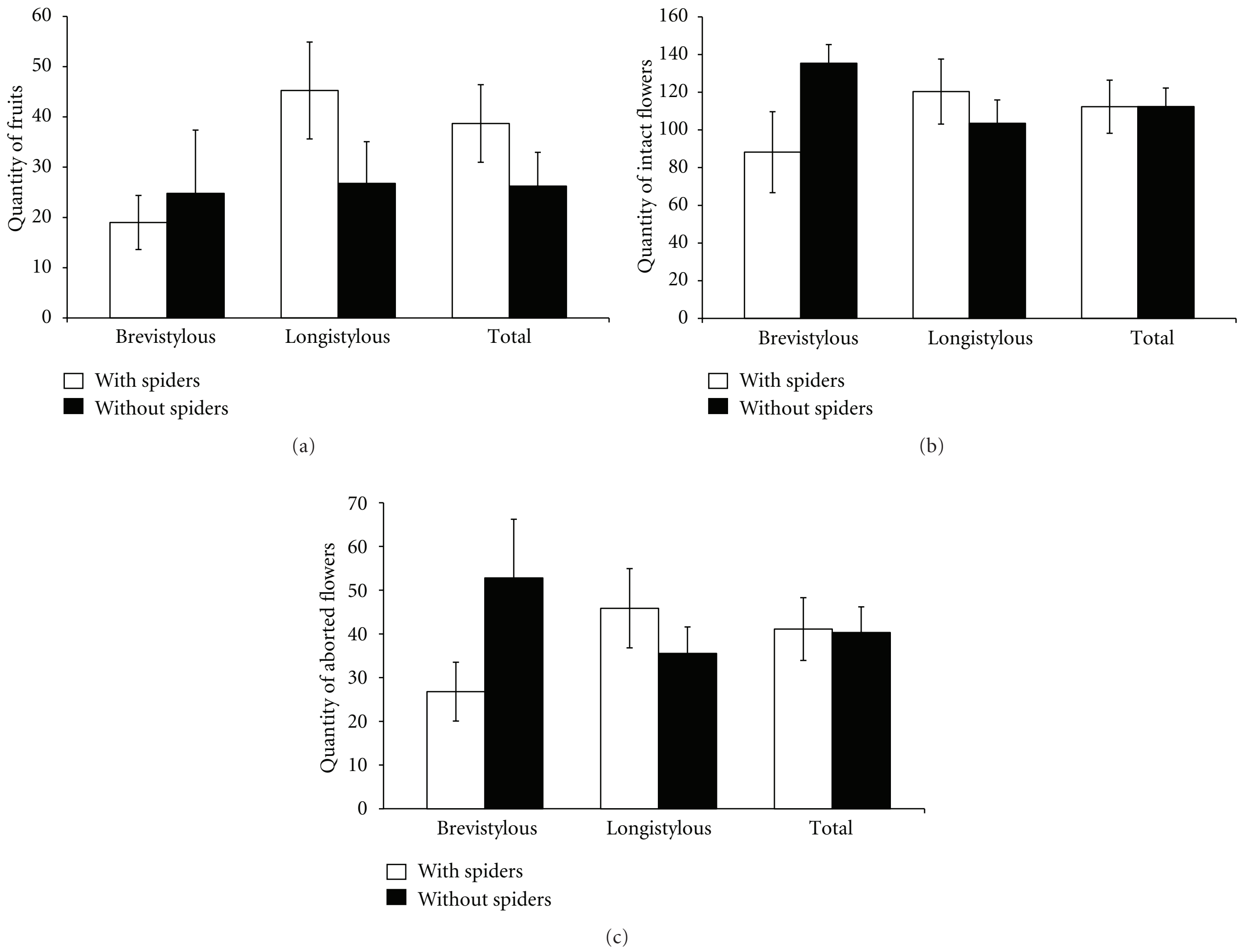


Figure 6: Quantity of fruits (a) and of intact (b) and aborted flowers (c) on brevistylous and longistylous inflorescences with and without spiders. Error bars represent ± SE.

Another important aspect is the presence of nectaries at the corolla base of the plant, which could attract other visitors to these resources, such as ants. The presence of ants culminates in the protection of the plant against herbivores action, reducing leaf area loss and increasing fruit set production [36]. Thus, multiple predators (ants and spiders) often have effects on their common prey populations that cannot be predicted by summing the effects of each predator at a time. Recently, [5] experimentally evaluated the effects of spiders and ants on herbivory and reproduction of *Q. multiflora* by dividing the trees in four experimental groups, depending on the presence or absence of ants and spiders. Results showed that the presence of ants reduced the abundance and richness of spiders, but spiders did not affect the abundance and richness of ants. Only the removal of ants resulted in a statistically significant increase in the herbivore abundance and richness. Herbivory, however, was also affected by spiders. In addition, authors found a significant interaction effect of ants and spiders on herbivory, indicating an emergent multiple predator effect. This study highlights the importance of evaluating the effect of the predator fauna as a whole and not only one specific group on herbivory. Moreover, the presence of ants on *P. rigida* as well as its ornithophilous pollination, as discussed before, is likely the cause, at least partially, of the low spider species richness and abundance on *P. rigida* inflorescences. Also, once spiders are usually solitary and aggressive toward both heterospecific and conspecific spiders [37] and the fact that many spider retreats occupied about one third of *P. rigida* inflorescence length, it would be possible that the paucity of spiders on *P. rigida* inflorescences is partly due to the lack of proximity tolerance among spiders. The presence of spiders on inflorescences may easily affect the plant reproductive success. Spiders can prey on phytophagous harmful to the floral structures and consequently reduce their abundance, a fact that lead to an increase in plant reproductive success [3]. Indeed, several studies have demonstrated that spiders may play an effective role in biological pest control [2, 38]. Although intra-guild predation and generalist spider habits could dilute their impact on phytophagous, the presence of a rich araneofauna and refuge availability may reduce the dilution effect, leading to the control and stability of prey populations by the spider assemblage [2, 28, 39]. Speculatively, it would be possible that longistylous inflorescences, due to the higher spider occurrence and architectural complexity, suffer less damage from phytophagous in their floral structures than brevistylous ones. The fact that cursorial guilds, such as ambushers and jumpers, are present mostly on longistylous inflorescences than on brevistylous ones also supports this conjecture, since cursorial spiders are the main predators in multispecies arthropod interactions [3] and probably the main agents of biological control [2]. Also, although there was a low spider number per longistylous inflorescence, synergic effects with other predators like ants might enhance the effect of spider presence on floral phytophagous. Albeit in this study we had not presented differences in number of fruits or intact and aborted flowers between the two *P. rigida* morphs, it would be possible that the effects of spider presence on longistylous inflorescences become evident on other *P. rigida* reproductive factors, such as seed viability and successful establishment of seedlings. Thus, it should be interesting for a future study to compare the viability of seeds and seedlings between the two morphs.

The fact that two floral morphs of the same plant species present a spider occurrence so distinct from each other is a really intriguing fact and worth further investigation. Moreover, the fact that the spiders are still present on almost all the longistylous inflorescences despite the unfavorable conditions for their establishment on *P. rigida*, such as the presence of ants and the ornithophilous pollination, reinforces the idea that longistylous inflorescences have some traits that enhance the habitat suitability for spiders. If plant architecture is the main factor for the spider presence differentiation between the two morphs, we might say that architectural modifications in inflorescences may alter the occurrence pattern of at least some predator groups. Thus, plant architecture variations could lead to bottom-up effects and influence the phytophagous abundance through modifications on predator occurrence pattern. Hence, would it be possible that a distinct architecture between two floral morphs may yield differential reproductive success due to variations on the phytophagous damage magnitude? Experimental studies with *P. rigida* and other heterostylous plant species, preferentially with higher spider abundance on their inflorescences, would eventually answer this question.

Disclosure

Three statistical packages were utilized for the data analysis. The authours chose Statistica 7.0 in order to perform nonparametric tests and Analysis of Variance, whereas for the Principal Component Analysis, they preferred Fitopac 2.1.2.85, since this statistical package is specific for Multivariate Analysis. The EstimateS 7.50 software is specific for species richness estimation, which is not performed either by Statistica or Fitopac packages. So, the software choice was solely based on the suitability of analysis properties from each statistical package in order to accomplish the research objectives.

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Research Article

Comparison of the Ant Assemblages in Three Phytophysionomies: Rocky Field, Secondary Forest, and Riparian Forest—A Case Study in the State Park of Ibitipoca, Brazil

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Ant assemblages are almost all related with the vegetation composition and so can provide us important information for conservation strategies, which are especially relevant to an environmentally protected area. We sampled the ant fauna in three different phytophysionomies in order to verify if the composition of ant species is different among the areas, especially because one of the areas is a Rocky Field and there is little information about the ant fauna in this habitat. A total of 8730 individuals were registered and an NMDS analysis showed that the ant assemblies are different at the three phytophysionomies (Rocky Field, Riparian Forest, and Secondary Forest). This study shows that the species that compose the ant assemblies in different phytophysionomies are a reflex of the environment, supporting the hypothesis that the vegetational composition results in different compositions in the ant assembly. Vegetal composition is determinant in the formation of the litter and consequently in the occurrence of ant species that depend on this layer of organic matter for nesting and foraging.

1. Introduction

Ants exert important effects in most ecosystems due to their abundance, population stability, and foraging activity [1, 2]. Some of their main activities are the nutrient cycling and control of other invertebrate populations [3]. They also participate actively in the composition of vegetation through seeds dissipation [4, 5], which gives them great importance in recovering degraded areas [6].

Besides their ecological importance, the Neotropical ants fauna are still little studied, especially if we consider the region of "Mata Atlântica" (Atlantic Forest) which occupies only 7% of its original area, according to the Ministry of Environment data [7]. Studies made in this biome indicate a high diversity of endemic species, which may comprise 50% of total species and 95% in certain groups [8, 9].

Actually in recent years, myrmecologists attention has been concerned essentially with ant communities, ant-plant relations, mutualisms, biomonitoring, biogeography, morphology and anatomy, genetics and cytogenetics, and taxonomy [10]. Ant species inventories made in Brazil are used to evaluate the conservation state of the environments, especially in fragmented areas, as the Atlantic Forest [11]. Also, according to Delabie et al. [10] perusal of recent papers indicates there are still new ant species to be described in Neotropical.

Inserted in the Atlantic Forest Biome, the State Park of Ibitipoca (PEIb) is classified in the category of "Extreme Biological Importance" because of endemism of some species, the relevance, the speleological singularity, and the diversity of habitats [12]. This park occupies an isolated hilly area from other areas of Rocky Field, presenting a distinctive flora of "Cadeia do Espinhaço" itself, being considered a disjunction concerning this Range [13].

PEIb presents significant diversity not only of vegetation, but also of fauna, landforms, soils, and microclimates [14] and covers two areas of regional vegetation, originally composed by semideciduous seasonal forests and "cerrados" [15]. It may be distinguished by five basic types of phytophysiognomies—altitude "cerrado", Rocky Fields, Riparian Forest, capon of forest, and an area of dense ombrophilous secondary forest.

The Rocky Fields are distinguished mainly by the grassland vegetation consisting of grass, herbs, and shrubs on outcrops of quartzitic rocks associated to shallow soils and high solar incidence [16]. The PEIb floristic studies indicate predominance of "candeia" specimens (*Vanillosmopsis erythropappa*).

The Semideciduous Secondary Forest covers an area of 90 hectares at the south of the PEIb and it is totally surrounded by woodlands, being named "Mata Grande." Due to the presence of anticlinal crests, this environment is greatly influenced by clouds, winds, and lightning [17]. There is marked abundance of epiphytic plants and lichens, with predominance of high trees (up to 25 m) [18].

The Riparian Forest in its extension mostly consists of shrubs patches that accompany the distribution of thicker soils, in slope conditions or concave lands. This subtype of vegetation is humid, with reduced wind action and remarkable presence of bromeliads and mosses and, in the edges or less shaded areas, many kinds of lichens [19].

Considering that the vegetation is a good predictor of the structure of community of ants [20–23], one could suppose that in different phytophysionomies the composition of ant species is equally distinct. In this context, a comparison of ant assemblies in three distinct phytophysionomies was accomplished—Rocky Field, Semideciduous Secondary Forest, and Riparian Forest—in the PEIb. The knowledge of how the ant species composition varies according to different characteristics of habitat provides important information for conservation strategies, which are especially relevant to an environmentally protected area.

2. Material and Methods

2.1. Area of Study. This study was conducted in the State Park of Ibitipoca—PEIb (21°40′44′′ S and 43°52′55′′ W) in the city of Lima Duarte, Minas Gerais, Brazil (Figure 1). The PEIb has approximately 1.488 ha with a mesothermic humid climate (Köppen classification), with dry winters, pleasant summers, and average annual temperature around 18.9°C. The influence of the relief over the climate is very important, because the altitude and topography are differentiated and the anticlinal crests in the PEIb stand out locally concerning the neighboring areas, leading to a differentiated climate in the area [17]. In the PEIb three phytophysionomies were sampled: Rocky Fields (RKF), Semideciduous Secondary Forest (SSF), and Riparian Forest (RPF).

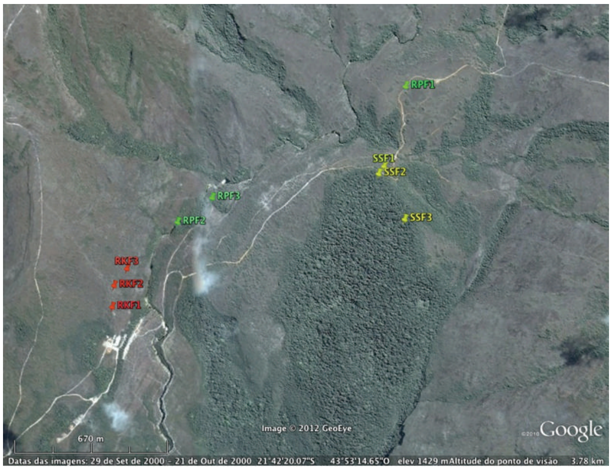

Figure 1: Parque Estadual do Ibitipoca (PEIb) in Minas Gerais State, Brazil. Red pins: Rocky Fields (RKF); Green pins: Riparian Forest (RPF); Yellow pins: Semidecidous Secondary Forest (SSF). Font: Google Earth, 2010.

2.2. Ants Sampling. In each of the three phytophysionomies, three quadrants of 800 m^2 each were established. The minimum distance between the quadrants within the same phytophysionomy was 50 m. The sampling of ants was accomplished monthly between July and December 2008.

In each quadrant three parallel transects were established, spaced from each other by 10 m. Along each transect the sampling points were determined apart from each other also by 10 m, in a total of 15 samples/quadrant. In each transect a different method was employed, as follows: honey and sardine attractive baits, pit-fall traps, and extraction in Berlese funnel of litter samples.

The baits contained 5 g of a mixed paste of honey and sardine (1 : 1 vol), distributed over paper tissues. The baits remained in the field for 60 min [24], after been collected for screening. Pitfall traps consisted of 500 mL plastic cups filled with 200 mL of water and liquid neutral detergent (10%). Pitfall traps remained in field for 24 h. Litter samples were standardized with a plastic grid of 0.25 m^2 on soil. The litter was put in Berlese funnel for 48 h for screening material.

In the quadrants of Rocky Fields, the pitfall traps and the litter samples were replaced by the attractive bait, considering the impossibility of using these methods, because litter is absent in the Rocky Fields and the installation of pitfalls under the rock is infeasible.

Collected ants were sorted, counted, and stored in alcohol 90%, recording phytophysionomy, method, and date of collect. After, a taxonomic identification was made under stereoscopic microscope (Leica), from dichotomous keys

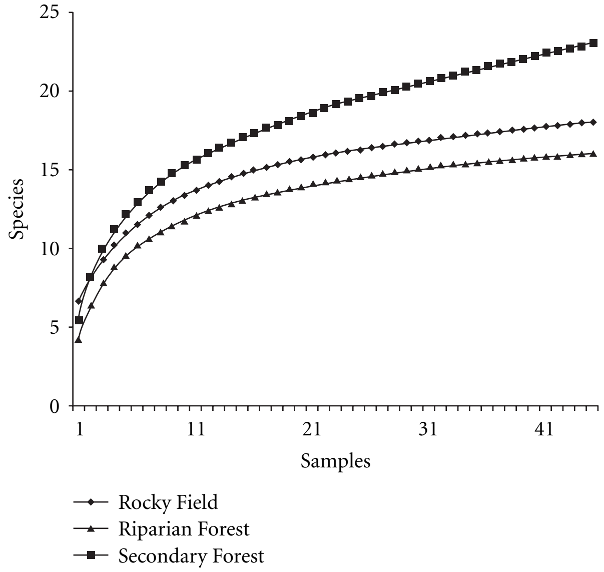

Figure 2: Sample-based rarefaction curves for the ant species at three phytophysionomies at Parque Estadual do Ibitipoca—Brazil. July–December, 2008.

[25, 26]. Ants were coded at genera level and separated in morph-species. Dr. Rodrigo Feitosa from the Museum of Zoology of São Paulo confirmed the species identification. The specimens were mounted and deposited in the thematic collection of MirmecoLab, ICB-UFJF (Campus Universitário, Cidade Universitária-s/n, Juiz de Fora-MG, CEP: 36036900).

2.3. Data Analysis. To evaluate the sampling effort, we constructed a rarefaction curve [27], using the program EstimateS [28]. Sample-based rarefaction curves indicate that sampling effort was significant for three phytophysionomies (Figure 2).

The content of five samples per method of collect was grouped to obtain a single sample for each transect in each quadrant. To compare the ant species composition from different phytophysionomies, we used multivariate analysis with the program PAST [29]. Data were organized in a binary matrix, considering the taxonomic level of genera (presence and absence) and submitted to nonmetric multidimensional ordination (NMDS). The dissimilarity between the phytophysiognomies was calculated through the Bray-Curtis index, which is less affected by the occurrence of rare species in the samples [30]. The stress index calculated by NMDS is a measure of goodness-of-fit [31].

Also a one-way analysis of similarity was applied (one-way ANOSIM), with 10 thousand permutations. This analysis compares the differences between the averages of the ranked similarities among the samples within and between the phytophysiognomies, verifying if there are significant differences in the composition of genera. ANOSIM generates a statistic R, which is a measure of dissimilarity between the areas. R values near zero indicate high similarity while R values near 1 indicate low similarity [32]. To calculate ANOSIM, the Bray-Curtis index was also used and each R value has its corresponding probability.

A similarity percentage test (SIMPER) was applied, which permits to determine which genera more contributed to discriminate among assemblies. SIMPER analysis provides a percentage of dissimilarity among the phytophysiognomies, presenting a percentage of contribution of each genera for such dissimilarity [31].

3. Results

A total of 8.730 individuals were collected, belonging to 46 species, 20 genera, and eight subfamilies: Ecitoninae, Ectatomminae, Heteroponerinae, Ponerinae, Formicinae, Dolichoderinae, Pseudomyrmecinae, and Myrmicinae, providing a list of ant species that occur in the PEIb (Table 1).

The greatest number of species was recorded for Riparian Forest, followed by Rocky Field and Secondary Forest, listing as exclusive species of Riparian Forest: *Pheidole* sp6, *Strumigenys louisianae*, *Brachymyrmex* sp2, *Paratrechina* sp1, and *Labidus* sp1. As unique species of Rocky Field are listed up: *Cephalotes pusillus*, *Brachymyrmex* sp3, *Camponotus genatus*, *Myrmelachista* sp2, *Myrmelachista* sp3, and *Pseudomyrmex* sp1; and in the Secondary Forest: *Brachymyrmex* sp1, *Myrmelachista* sp1, and *Hypoponera foreli* (Table 1).

Among the 19 ant species shared within the three studied areas, the most representative genera were *Pheidole* (7 species), *Camponotus* (4 species), and *Hypoponera* (3 species). We highlight the *Camponotus* absence in the Secondary Forest and the exclusive occurrence of each one of the three *Brachymyrmex* species in each phytophysionomy (Table 1).

Ant species composition in the three phytophysionomies differed significantly. (ANOSIM, $R = 0.48$, $P = 0.0001$), being more similar to the samples belonging to the same phytophysionomies (Figure 3, Table 2). The ordination NMDS indicates a stress value of 0.16, with the coordinates 1 and 2 explaining 48% and 26% of data variation, respectively. Actually, the greatest values of dissimilarity were verified between the Secondary Forest and the Rocky Fields (Table 3), being their samples, respectively, separated by coordinate 1.

According to the SIMPER test, the genera that most contributed for the dissimilarity among the phytophysionomies were *Crematogaster* and *Myrmelachista* which are responsible for 66.58% of the variation of species composition among the phytophysionomies (Table 4).

4. Discussion

The phytophysionomies showed differences in the composition of ant species, especially between SSF and RKF (Figure 3), evidencing the relationship between the vegetation and the ant fauna.

The SSF presents larger diversity of vegetal species, with genera of the families Rubiaceae, Lauraceae, Myrtaceae, Euphorbiaceae, Nyctaginaceae, Melastomataceae, Annonaceae, Palmae, Apocynaceae, and Monimiaceae [19]. This

Table 1: Relative frequency of occurrence of ant species in the three phytophysionomies in the Parque Estadual do Ibitipoca, Brazil. July–December, 2008.

Species	Rocky Field	Riparian Forest	Secondary Forest
Myrmicinae			
Acromyrmex aspersus F. Smith, 1858	0.00	92.29	7.71
Acromyrmex hispidus Santschi, 1925	20.08	78.74	1.18
Apterostigma (gr. *pilosum)* sp1 Mayr	20.00	40.00	40.00
Apterostigma sp2 Mayr	94.12	0.00	5.88
Cephalotes pavonii Latreille, 1809	93.52	6.48	0.00
Cephalotes pusillus Klung, 1824	100.00	0.00	0.00
Crematogaster sericea Forel, 1912	98.46	1.50	0.05
Octostruma balzani Emery, 1894	0.00	37.50	62.50
Octostruma rugifera Mayr, 1887	0.00	50.00	50.00
Pheidole obscurithorax Forel, 1985	77.78	15.56	6.67
Pheidole radoskowskii Mayr, 1884	44.32	5.68	50.00
Pheidole sp1 Westwood	21.90	59.65	18.45
Pheidole sp2 Westwood	39.20	14.20	46.60
Pheidole sp3 Westwood	48.88	26.85	24.27
Pheidole sp4 Westwood	33.33	18.52	48.15
Pheidole sp5 Westwood	60.14	17.39	22.46
Pheidole sp6 Westwood	0.00	100.00	0.00
Solenopsis sp1 Westwood	1.03	97.94	1.03
Solenopsis sp2 Westwood	38.96	57.14	3.90
Strumigenys louisianae Roger, 1863	0.00	100.00	0.00
Wasmannia affinis Santschi, 1929	27.78	50.00	22.22
Wasmannia auropunctata Roger, 1863	19.28	57.83	22.89
Formicinae			
Brachymyrmex sp1 Mayr	0.00	0.00	100.00
Brachymyrmex sp2 Mayr	0.00	100.00	0.00
Brachymyrmex sp3 Mayr	100.00	0.00	0.00
Camponotus crassus Mayr, 1862	92.14	7.86	0.00
Camponotus genatus Santschi, 1922	100.00	0.00	0.00
Camponotus melanoticus Emery, 1894	59.46	40.54	0.00
Camponotus pr *bonariensis* Mayr, 1868	60.00	40.00	0.00
Camponotus renggeri Emery, 1894	78.02	21.98	0.00
Myrmelachista sp1 Roger	0.00	0.00	100.00
Myrmelachista sp2 Roger	100.00	0.00	0.00
Myrmelachista sp3 Roger	100.00	0.00	0.00
Paratrechina sp1 Motschoulsky	0.00	100.00	0.00
Ectatominae			
Ectatomma edentatum Roger, 1863	58.23	40.51	1.27
Heteroponerinae			
Heteroponera dentinodis Mayr, 1887	0.00	18.75	81.25
Heteroponera inemis Emery, 1894	0.00	5.56	94.44
Ponerinae			
Hypoponera foreli Mayr, 1887	0.00	0.00	100.00
Hypoponera sp1 Santschi	1.75	8.77	89.47
Hypoponera sp2 Santschi	25.00	25.00	50.00
Hypoponera sp3 Santschi	0.00	7.32	92.68
Pachycondyla striata Smith, 1858	22.22	50.00	27.78
Ecitoninae			
Labidus sp1 Jurine	0.00	100.00	0.00

Table 1: Continued.

Species	Rocky Field	Riparian Forest	Secondary Forest
Dolichoderinae			
Linepithema cerradense Wild, 2007	89.61	7.79	2.60
Linepithema pulex Wild, 2007	9.35	90.65	0.00
Pseudomyrmicinae			
Pseudomyrmex sp1 Lund	100.00	0.00	0.00

Table 2: Comparisons ANOSIM paired of the composition of ant species in the three phytophysiognomies sampled in the Parque Estadual do Ibitipoca, Brazil. July–December, 2008.

	Riparian Forest	Secondary Forest	Rocky Field
Riparian Forest	—	0.257 ($P = 0.0003$)	0.435 ($P = 0.0003$)
Secondary Forest		—	0.747 ($P = 0.0003$)
Rocky Field			—

Table 3: Dissimilarity values (SIMPER) between the three phytophysiognomies sampled in the Parque Estadual do Ibitipoca, Brazil. July–December, 2008.

	Riparian Forest	Secondary Forest	Rocky Field
Riparian Forest	—	83.45	86.31
Secondary Forest		—	88.61
Rocky Field			—

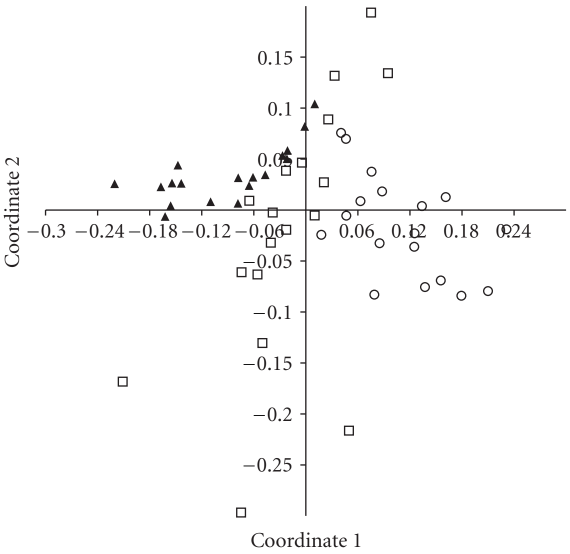

Figure 3: Nonmetric multidimensional ordination (NMDS) of ant species composition in three phytophysionomies sampled in the Parque Estadual do Ibitipoca, Brazil. July–December, 2008. Secondary Forest (triangles), Riparian Forest (squares), and Rocky Field (circles). Stress value = 0.16.

vegetation composition promotes the litter formation and, consequently the occurrence of cryptic ant species that depend on this layer to their nesting and foraging [33].

Actually we sampled seven and six cryptic ant species at SSF and RPF, respectively, while there are just four cryptic ant species at RKF. We pointed out that among these four cryptic species at RKF, two of them are arboricolous (*Myrmelachista*) [34], so they are not litter-dependent for nesting or foraging.

The Rocky Field presents characteristics completely different from the other areas. With a rocky soil, this open environment has a predominance of small trees with extra floral nectaries, especially of the genera *Vanillosmopsis* ("candeia"). Also the lack of nearby water bodies makes this phytophysionomy a hostile environment. According to Campos [16], rocky and sand exposed at the top of these fields are among the most extreme combinations of an environment. Mountainous areas, on which are found the rocky fields, are comparable to islands separated by very different ecological conditions [35]. The occurrence of arboreal ant species that present association with plants (*Crematogaster, Cephalotes, Pheidole, Camponotus, Myrmelachista, Linepithema,* and *Pseudomyrmex*) is a reflex of Rocky Field characteristics.

We emphasize the high frequency of *Camponotus* in the Rocky Field in contrast to its absence in the Secondary Forest. *Camponotus* is cited as the most frequent in open habitats such as sandbanks [36], "cerrado" [37–39], and "caatinga" [40]. This study includes Rocky Field as a habitat that allows to its occurrence. The absence of *Camponotus* in the Secondary Forest could be related to the achievement of collects exclusively in soil, combined with the high scale of the vegetation in this area.

Besides *Camponotus, Brachymyrmex* is also noteworthy, given the observed spatial segregation in which each of the three species was exclusively sampled in one of the areas. This spatial segregation can be explained given the high level of aggression recorded for the genera, even in intraspecific interactions [41].

For Riparian Forests the values of dissimilarity are near 50% and can be considered as a transition range between the two other phytophysiognomies, agreeing with the spatial location of this habitat in the PEIb (Figure 1) and with the presence of specialist (e.g., *Acromyrmex, Labidus*), invasive (e.g., *Solenopsis, Paratrechina*), and cryptic ant species (*Strumigenys*).

This study shows that the species that compose the ant assemblies in different phytophysiognomies are a reflex of the environment, especially of the plant species, supporting the hypothesis that differences in the vegetational composition result in different compositions in the ant assembly.

Also the vegetational composition is determinant in the formation of the litter and consequently in the occurrence of ant species that depend on this layer of organic matter for

Table 4: Cumulative contribution of ant genera for the dissimilarities among the phytophysionomies (SIMPER) sampled at Parque Estadual do Ibitipoca, Brazil. July–December, 2008.

Genus	Cumulative contribution %	Average abundance		
		Rocky Field	Riparian Forest	Secondary Forest
Crematogaster	42.64	1.78	0.05	0
Myrmelachista	66.58	0	1.78	0.05
Pheidole	80.35	0.05	0.27	2.78
Acromyrmex	93.13	3.83	0.33	0.11
Camponotus	96.92	0	0.22	2.83
Linepithema	98.83	0.22	0.5	0.27
Solenopsis	99.41	0.88	0	0.05
Wasmannia	99.68	0.11	0.11	0.27
Heteroponera	99.85	0	0.05	0
Strumigenys	100	0.11	0	0

nesting and foraging as the cryptic ant species [42]. Obtained data suggest that determination of ant fauna in the Secondary Forest and Riparian Forest is dependent of the conditions and resources provided by these phytophysiognomies, for instance, the presence of litter, shaded areas, and high trees. Unlike, in the Rocky Field, it is expected that the competition is the most important factor in determining the species that compose the assembly, considering the absence of litter, high insolation, and scarcity of resources.

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Research Article

Biology of *Omaspides pallidipennis* Boheman, 1854 (Coleoptera: Chrysomelidae: Cassidinae)

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The biology and the feeding habits of the subsocial species *Omaspides pallidipennis* were studied at the Floresta Nacional de Passa Quatro, MG, Brazil, during the period from October 2010 to April 2011. The species was bivoltine, beginning its reproductive and food cycle in October (spring) and seeking its diapause sites in April (autumn). The juveniles took 54.4 days on average to complete their development, a period in which the female remained close to offspring, only feeding during the larval stage of the juveniles. It is a monophagous species, feeding only on *Ipomoea alba* Linnaeus (Convolvulaceae). In the first cycle, the average number of eggs was 55.7 ± 15.5 eggs per egg cluster ($n = 1,837$ eggs in 33 clusters) and in the second it was 61.6 ± 14.2 eggs per egg cluster ($n = 5,607$ eggs in 91 clusters). Oviposition peaks were observed in the months of November and February. The average durations of the incubation period and the larval and the pupal development in the first cycle were 19.2 ± 1.4; 26.0 ± 1.5; 8.7 ± 0.8 days, respectively. In the second cycle they wrere 16.7 ± 1.4; 27.0 ± 2.4; 10.2 ± 1.5 days, respectively.

1. Introduction

The family Chrysomelidae is one of the largest among the insects of the order Coleoptera [1]. Due to its diversity of representatives it is subdivided into 19 subfamilies [2]. Among these Cassidinae stands out for being the second largest in number of species (ca. 6,000 species), with approximately 16% of the diversity [3]. Its representatives also stand out for having unique morphological, ecological and biological characteristics [4]. However, an evident problem that exists regarding that subfamily is the shortage of information regarding the biology of many of its species. Although the majority is solitary, various species are subsocial. The study of those characteristics can explain the determination of the sequence and exact number of transitions among the way of life of the solitary, gregarious, and subsocial species [5]. Moreover, to know the relationship between the performance of the offspring and the egg laying preference, it is essential to understand the population dynamics of herbivore insects, as well as their distribution [6].

The majority of existing research on Cassidinae about the biology of the species, solitary or subsocial, was conducted in laboratory [7–10]. In field, the biology of subsocial species is described, minutely, for *Acromis sparsa* Boheman, 1854 (see, e.g., [11, 12]) and *Omaspides tricolorata* Boheman, 1854 [13, 14]. However, the number of species that exhibiting that behavior is much higher (16 species described, for the Stolaini and Eugenysini tribes) and should increase, due higher number of researchers working with this theme.

For the subsocial species *Omaspides pallidipennis* Boheman, 1854, no data was found on its biology. Information about the description of the pupa and adults were given by Costa Lima [15], also registering the presence of the subsocial behavior [11, 15–18]. As for its distribution in Brazil, the species is found in the states of Espírito Santo, Minas Gerais, Paraná, Rio Grande do Sul, Rio de Janeiro, Santa Catarina, and São Paulo [19], in environment of Atlantic forest, riparian forest, and savanna (Fernando Frieiro-Costa, personal information). In relation to the host plant, information is also scarce. Few information exists of *Ipomoea alba* Linnaeus, 1753 (Convolvulaceae) as host plant [19, 20]. Although most of the subsocial Cassidinae have been observed in only one type of host plant, some species can be found on different host plants genus. For *O. pallidipennis,* this fact has not been observed (Fernando Frieiro-Costa, personal information).

The objective of the present work was describe the biology of *Omaspides pallidipennis* Boheman, 1854 (Coleoptera: Chrysomelidae: Cassidinae) and its relation with host plant, in a natural environment in the Atlantic Forest biome.

2. Material and Methods

2.1. Study Area. The research was conducted in the Floresta Nacional (FLONA) de Passa Quatro, Municipal district of Passa Quatro, Minas Gerais State, Brazil (22° 23′ S, 44° 56′ O); altitude of 900 m; 335 ha), in an Atlantic Forest recovery area. The Conservation Unit (CU) contains roads that are used by tourists for visitation and by the guards for local patrols. The study was conducted on the host plants that grew on the edge of one of those roadsides.

The vegetation of CU is characterized by the insertion of a Semidecidual Seasonal Forest in the Atlantic Forest Biome, with a prevalence of planted plant coverings of pine, araucaria, and eucalyptus. Regionally, besides the Semidecidual Seasonal Forest, the Dense Ombrophylous Forest and Mixed Ombrophylous Forest typologies are found in the area [21]. The climate of the area, according to the Köppen classification, is Cwa-moderate temperatures with hot and rainy summers and dry winters. The climatic data were supplied by the National Institute of Meteorology (INMET) and presented an average temperature of 21.4°C, with precipitation and relative humidity of 291.9 mm and 76%, respectively, for the first life cycle of the species (October/January). For the second cycle (February/April) the temperature, precipitation, and relative humidity averages were 21.6°C, 116.9 mm, and 75%, respectively.

2.2. Biological Study of O. pallidipennis. The population of *O. pallidipennis* was observed daily, in the morning and in the afternoon (at alternate times), during the period between the months of October 2010 to April 2011. In this period 170 females with egg masses were accompanied and marked. The females received a mark on their elytron, facilitating the observation of parental care, of number of eggs deposited in each cycle, and of the development duration of the juvenile stages. For the marking of the females the Frieiro-Costa and Vasconcellos-Neto methodology was used [14]. Photographs of the egg masses, when the guardian was not over them, facilitated the obtaining of the average number of eggs. The oviposition and eclosion times were logged. Daylight saving time was not taken into account at any time.

2.3. Host Plant. The latescent *I. alba* vine frequently occurs in forest borders. It can also be found in crop areas, where it is a serious competitor of cultivated plants [22]. The flowers are solitary or gathered in groups, with a white or pinkish coloration [23–26]. In the lamina/petiole intersection there are extrafloral nectaries (EFNs) which are constantly visited by various insect species, especially ants. In Brazil this plant can be found in the states of Bahia, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul, and Ceará [23].

2.4. Statistical Analysis. The data were submitted to the Kolmogorov-Smirnov test, to verify the distribution type, being expressed as the average ± standard deviation (SD). To compare the data of number of eggs and developmental time of immatures between one cycle and another, the Student's *t*-test was used for normal distribution data and the Mann-Whitney test for free distribution. For these analysis the Bioestat version 5.3 software was used [27].

3. Results and Discussion

3.1. General Aspects of Biology of O. pallidipennis. Bivoltine Coleoptera, *O. pallidipennis* began their reproductive and feeding activities in October (spring) and they sought the diapause sites in the middle of April (autumn). During the whole cycle the juveniles only received care by the female that protected them from any imminent danger.

Species of subsocial tropical Cassidinae, like *O. pallidipennis*, *O. tricolorata* [14] and *Omaspides brunneosignata* Boheman, 1854, do not usually present more than two annual generations, because they spend much time and energy taking care of a single group of offspring. For not being exposed to the seasonal extremes that impede reproduction and growth, tropical and subtropical Cassidinae, subsocial or not, can present a greater number of generations [28, 29], if compared to temperate region species that are usually univoltine [30, 31]. Nevertheless, they are exposed to the alterations of the dry and rainy stations, related to the adequate availability of food [32]. In some of those tropical species, the synchronization of the life cycle with the variable conditions is enabled through the diapause [32].

In the FLONA of Passa Quatro, *O. pallidipennis* presented monophagous habits. Adults as well as juveniles only fed on *I. alba.* Although other plants of the same family and same genus have been found in the CU, those Cassidinae were never observed on another host plant species. Besides *O. pallidipennis,* egg masses and adults of the solitary species *Chelymorpha inflata* Boheman, 1854 (Cassidinae: Stolaini) were found also feeding on *I. alba.* At no time were both species observed feeding on the same leaf. Besides *C. inflata,* grasshoppers and Chrysomelinae and Lepidoptera larvae were found feeding on the leaves of the chosen host.

I. alba was observed in FLONA of Passa Quatro, in an open field area as well as roadside. The specimens of the host plant remained under direct sunlight most of the day, with few shaded portions.

3.2. Immature Stages. These insects are holometabolic, their cycle being completed in approximately two months (54.4 days on average, from egg to adult).

3.2.1. Eggs. The egg clusters of *O. pallidipennis* presents a diamond-shaped format that, with elongated eggs, approximately 2.8 times longer than their highest width and without any covering (Figure 1(a)). When recently laid they

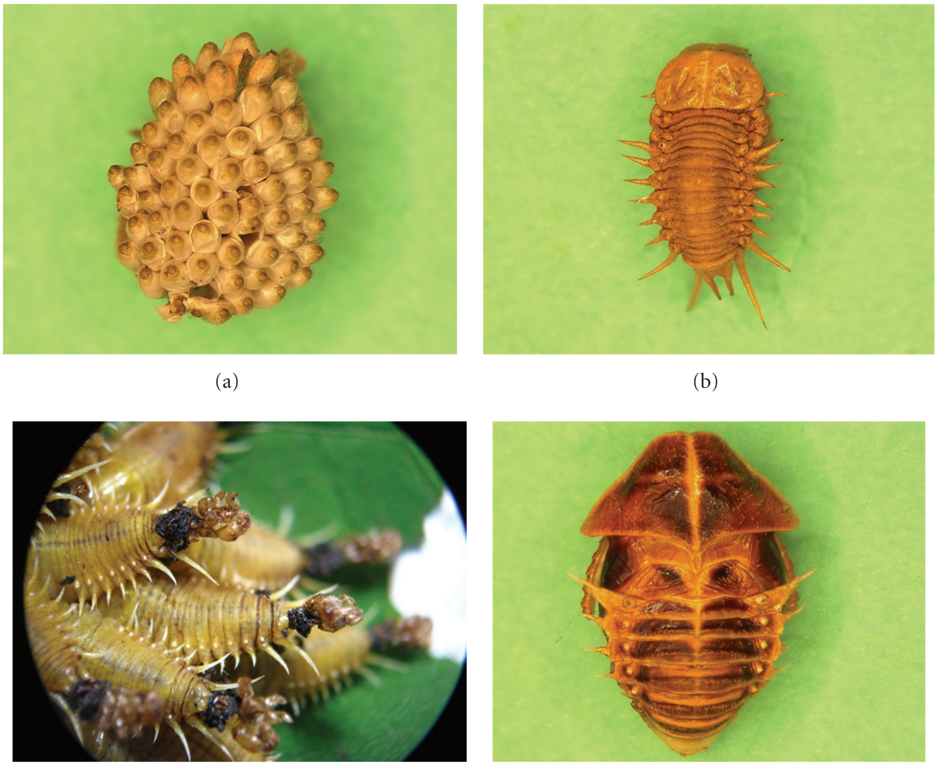
(a) (b)
(c) (d)

Figure 1: Immature stages of *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae). (a) Egg cluster, (b) dorsal view of last instar larvae, (c) exuvial-fecal shield, (d) pupae in dorsal view. Photos: (a), (b), and (d): Flávia Fernandes.

presented an amber coloration (Figure 2(a)) later becoming straw-yellow as the hardening of the chorion occurred (Figure 2(b)). That difference in the coloration allowed the distinction of the oldest egg clusters from the most recent. In the first cycle (October to December) the oviposition presented, on average, 55.7±15.5 eggs/egg clusters ($n = 1{,}837$ eggs in 33 clusters; range 12–80 eggs), and in the second cycle (February to April) the average corresponded to 61.6 ± 14.2 eggs/egg clusters ($n = 5{,}607$ eggs in 91 clusters; range 13–80 eggs). The ratio between the number of egg masses in the first and second cycles was significantly different ($U = 1106.00$; $P = 0.0253$). The factors for this difference can be attributed to the disparity existent between one female and another regarding their physiological and nutritional state, the nutritional state of the host plant leaves (young leaves, under growth have higher level of nitrogen than the mature leaf) [33], and to the abiotic factors, as the temperature. In many insects, the production of eggs is controlled by one or more hormones produced in the *corpora allata*, that control the initial stages of oogenesis and the yolk deposition. Factors such as the temperature can act on these structures, thus affecting the egg production [34].

Subsocial species of the same genus, like *O. tricolorata* [14] and *Omaspides convexicollis* Spaeth, 1909 [35], also present a large number of eggs per cluster (average of 55.1 and 48.8, resp.), if compared to other non-subsocial species such as *Anacassis dubia* Boheman, 1854 with an average of 9.1 eggs per cluster and *Anacassis languida* Boheman, 1854 with an average of 6.7 eggs per cluster [9, 36]. The female of *Charidotis punctatostriata* Boheman, 1856 produces, annually, an average of 235.5 ± 41 eggs per female [8], a quantity that can be attributed to the high reproductive effort due to the semelparity presented.

The large number of eggs in subsocial species can also be explained by the high reproductive effort, because they spend most of their time investing in the defense of the offspring and in resource allocation, instead of going through various ovipositions. However, the subsociality is one of several adaptations aimed at facing adverse conditions [37]. Unlike the physical protection provided to the eggs by the mother, as in *Acromis sparsa* Boheman, 1854 [38], the non-subsocial Cassidinae can make use of different adaptations, such as the protection of the eggs through an ootheca [39–41] and ooótheca and feces [42] or a gelatinous matrix with feces, as in *Hemisphaerota cyanea* Say, 1824 [43], thus making access more difficult for the natural enemies.

Regarding the egg laying site, the ovipositions of *O. pallidipennis* were all deposited on the abaxial surface of *I. alba*, a behavior also present in other subsocial [11, 13, 44, 45] and non-subsocial species [39, 40]. For the species *Gratiana spadicea* Klug, 1829 and *O. tricolorata* this behavioral pattern is related to the temperature [14, 46]. Although it had not been measured, the temperature was also pointed to as a decisive factor of this behavior, because

(a) (b)

Figure 2: *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae) female (a) on recently laid egg cluster (b) after a few days. A fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

the majority of the host plant leaves were under direct sunlight several hours a day.

The choice of the female for the egg laying site is an important factor for the growth and the survival of their larvae [47]. When ovipositing, the female should consider an appropriate place for the development of the juveniles, thus maximizing their adaptive value. Factors such as the predation risk [47, 48], host plant quality or quantity [33, 49], larval mobility [50], and the intraspecific and interspecific competition [51] should be considered. Of the 170 egg masses observed, 159 allowed to know the oviposition site with certainty. Of these, 116 (73%) were found along the midrib and 43 (27%) in other parts of the leaf blade, no egg masses being placed in the proximal half of the petiole. That preference to oviposit in the distal portions can be explained by the presence of predator ants that constantly visited host plant EFNs. Among them several ants of the genus *Pseudomyrmex* sp. (Formicidae) and *Crematogaster* sp. (Formicidae) preying on eggs and larvae were found. The oviposition preference on the host plant was not altered by the intraspecific competition, not finding more than one egg mass of the species or of other Cassidinae species on the same leaf.

The oviposition peaks occurred during the months of November and February, not observing any new egg masses, in December, January, and April. The average of incubation period of the eggs was 19.2 ± 1.4 days ($n = 31$ offspring) for the first cycle and 16.7 ± 1.4 days ($n = 71$ offspring) for the second cycle (Table 1). The incubation time differed significantly in the two cycles ($U = 239.00$; $P < 0.0001$). Characteristics such as abiotic factor variations can explain such difference. In *Metriona elatior* Klug, 1829 the average incubation time of the eggs is lower at 30°C (5.6 days) than at 20°C (11.3 days) [52]. Another factor to be considered is the quality and the quantity of the host plant that can alter nutrient acquisition, thus interfering in the production of eggs [53]. However, more research is necessary to explain these characteristics.

During the biological cycles, three females oviposited twice during the same cycle. In all those cases their first oviposition had been preyed upon. The time spent between one oviposition and the other varied from 1 to 19 days.

Table 1: Duration of the developmental immature stages of *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae), for the first and second cycle in a fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

	First cycle	Second cycle
	Mean ± SD	Mean ± SD
Egg	19.2 ± 1.4 ($n = 31$)	16.7 ± 1.4 ($n = 71$)
Larvae	26.0 ± 1.5 ($n = 19$)	27.0 ± 2.4 ($n = 35$)
Pupae	8.7 ± 0.8 ($n = 20$)	10.2 ± 1.5 ($n = 30$)
Total time	54.3 ± 9.0	54.4 ± 8.7

3.2.2. Larvae. The larvae of *O. pallidipennis* are light yellow, presenting a slightly dorsal-ventrally flat body. There are nine pairs of lateral scoli and a caudal furcae (Figure 1(b)) where the exuvial-fecal shield is attached [18] (Figure 1(c)). In some species of Cassidinae *s.str.*, this structure works as physical protection against dissection and predation [54, 55]. A chemical defense function, through compounds that are present in this attachment, is evidenced, also, in other species [56–58]. *Eurypedus nigrosignatus* Boheman, 1854 (Cassidinae: Physonotini) obtains those chemical compounds from its host plant *Cordia curassavica* (Jacques) Roemer and Schultes [59]. Studies evidence that these structures have been shown to be efficient against some natural enemies, but not against others. In *Cassida rubiginosa* Müller, 1776 the exuvial-fecal shield was effective against *Formica exsectoides*, Forel 1886 (Hymenoptera: Formicidae) [54] but not against *Polistes dominulus* Christ, 1791 (Hymenoptera: Vespidae) [60]. The fecal shield was also not effective for *Chelymorpha reimoseri* Spaeth, 1928 against *Polistes* sp. and *Piaya cayana* Linnaeus, 1766 (Cuculiformes: Coccyzidae) [61]. However, in *H. cyanea*, the fecal attachment was efficient against the coccinellid *Cycloneda sanguinea* Linnaeus, 1763 and the hemipteran *Stiretrus anchorago* Fabricius, 1775 but not

Figure 3: Leaf with signs of herbivory caused by *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae) in first stages. A fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

against *Calleida viridipennis* Say, 1823 (Coleoptera: Carabidae) [43].

In relation to the scoli, Eisner et al. [54] found evidences in *C. rubiginosa* that they act in the defense, because when they are touched, the larvae respond by quickly raising their fecal attachment.

Most of the Cassidinae larvae seem to have five development stages, like *O. pallidipennis*, *O. tricolorata* [14], *Cassida obtusata* Boheman, 1854 [62], and *M. elatior* [10]. However, some species present wide variation in the larval stages [3], arriving in *Chelobasis perplexa* Baly, 1858 (Hispinae *s.str.*) at eight development stages. That determination of the number of stages can be made through the measurement of the cephalic capsule [9, 63] or by counting the accumulated exuviae in the exuvial-fecal shield [14].

Soon after eclosion, the larvae begin to feed around the egg mass, moving towards the distal end of the leaf. In all of the larval stages feeding on the borders of the leaf towards the petiole was always observed. In the first stages, "they scraped" the parts between the ribbing, leaving the leaf with lacy aspect (Figure 3). Starting from the third stage, they fed on the whole leaf (primary and secondary ribs and petiole), changing to another leaf only when the previous was totally eaten. The larvae feed from the abaxial surface, as well as the adaxial surface, always joining after the feeding in cycloalexy, a form of gregariousness [64]. The larval gregariousness provides some advantages to the initial stage larvae, such as ease of feeding, economic use of restricted resource and group protection against their natural enemies [65, 66] thus not having interference of the intraspecific competition, as already mentioned, in the choice of the egg laying site for the female. During the whole developmental period of the juveniles, the female was only observed just feeding when the offspring were in the larval stage. At the end of the fifth stage, the larvae moved via the plant stem and were positioned in a clustered, imbricated manner, fastening the end portion of the abdomen to the branch, to then pupate (Figure 4).

The larval stage is the longest juvenile stage. For the first cycle, the larval development was 26.0 ± 1.5 days (n = 19 offspring), counted from eclosion to reaching the pupal stage. In the second cycle the duration was 27.0 ± 2.4 days ($n = 35$ offspring; Table 1). The n sample corresponds to the group of larvae that reached the pupal stage. The time of larval development among the two cycles did not show significant difference (t-test, $P = 0.0555$; df = 50.69).

Figure 4: Imbricated pupae of *Omaspides pallidipennis* Boheman, 1854 in stem of its host plant *Ipomoea alba* L. (Convolvulaceae). A fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

During the research, offsprings were seen with number of visibly smaller individuals. It can be considered another factor, besides the predation. Because the *O. pallidipennis* host plant was under constant sunlight exposure, it is possible that death by dehydration had occurred. Gandolfo et al. [52] reared *M. elatior* under different temperatures (20°C, 25°C, and 30°C) and their juveniles had faster development at higher temperatures. However, at 30°C the larvae suffered damage, not reaching the pupal stage. Frieiro-Costa and Vasconcellos-Neto [14] suggest that the larvae of *O. tricolorata* exposed to high temperatures can dehydrate and die.

3.2.3. Pupae. Soon after reaching the pupal stage they presented yellowish coloration, becoming yellowish brown with dispersed dark patches on the body after a period of 24 hours (Figures 1(d) and 4). As in the *A. languida* [36] species *O. pallidipennis* did not retain the exuvial-fecal shield at pupation. However, there are Cassidinae species that keep the exuvial-fecal attachment [67] or only the exuviae [68].

The pupal stage was the shortest of the development stages. In the first cycle, the duration was 8.7 ± 0.8 days (n = 20 offspring), presenting an average of 10.2 ± 1.5 days (n = 30 offspring) for the following cycle (Table 1). The difference in the time of development between the cycles was highly significant (t-test, $P < 0.0001$; df = 45.97), a reason that can be attributed here, as well as in the incubation period, to the variation of the abiotic factors. In the duration of the pupal development time, the larval stage group individuals that reached the subsequent stage were used as a basis. The prepupal period was not considered due to the short duration of that stage, which did not allow precise verification.

Of 43 studied groups, 35 pupated on the stem, and seven of these pupated on plants other than the host, which were support for *I. alba*. The eight groups remained pupated on the abaxial leaf surface. Of the groups, 19 were found pupated in areas under sunlight and the others in shaded locations. When the pupas stayed under direct sunlight, they protruded out, probably to increase the air circulation among them. High temperatures can hinder or impede the development of juvenile stages [52].

3.3. Adults. The adults are gregarious and they show no apparent sexual dimorphism. Upon emergence, the elytra and pronotum were a translucent yellow color, becoming straw-yellow after total sclerotization, that occurred in approximately seven days. During this period the female stayed close to juveniles on the abaxial leaf surface of the host plant. In *H. cyanea*, the adult, when emerging, was under its exuvial-fecal shield until total sclerotization of the elytra [43]. Recently emerged adults were not found mating.

Juveniles feeding started after about seven days. The adults started feeding from the edges of the *I. alba* leaf or preexisting holes in the leaf blade.

This paper explains the importance of observational studies in the field to understand the biology and ecology of the species. Subsocial Cassidinae provide excellent study material, because they are easily observed since they remain restricted to the development site of the juveniles throughout their development. However, further research should be conducted to further elucidate the relationship between subsocial or non-subsocial Cassidinae and their host plants.

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Research Article

Accidental Fire in the Cerrado: Its Impact on Communities of Caterpillars on Two Species of *Erythroxylum*

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Among the mechanisms that influence herbivorous insects, fires, a very frequent historical phenomenon in the cerrado, appear to be an important modifying influence on lepidopteran communities. The purpose of this study was to compare the richness, abundance, frequency, and composition of species of caterpillars in two adjacent areas of cerrado *sensu stricto*, one recently burned and one unburned since 1994, on the experimental farm "Fazenda Água Limpa" (FAL) (15°55′S and 47°55′W), DF, Brazil. Caterpillars were surveyed on two plant species, genus *Erythroxylum: E. deciduum* A. St.-Hil. and *E. tortuosum* Mart. (Erythroxylaceae). We inspected a total of 4,196 plants in both areas, and 972 caterpillars were found on 13.3% of these plants. The number of plants with caterpillars (frequency) differed significantly between the areas. The results indicate that recent and accidental fires have a positive effect on the abundance of caterpillars up to one year postfire, increase the frequency of caterpillars associated with *Erythroxylum* species in the cerrado and do not affect the richness of caterpillars on these plants. Moreover, the fires change the species composition of caterpillars by promoting an increase in rare or opportunistic species.

1. Introduction

Systems represented by the associations of plants and insects include more than one-half of the world's multicellular species. The impacts of disturbances, anthropogenic or otherwise, affect the characteristics of communities of herbivorous insects in any biome worldwide [1]. There is strong evidence that these disturbances result in complex changes in the interactions between plants and herbivores [2]. Fires affect communities of herbivorous insects and provide opportunities for changes in species richness, abundance and species composition in space and time [3]. Among herbivores, Lepidoptera can serve as good indicators of environmental changes caused by these disturbances in certain habitats [4].

Fires in the cerrado are a natural phenomenon of recognized ecological importance [5] and occur during the dry season, from May to September [6, 7]. The effects of fire on the structure, composition and diversity of plants in the cerrado are far more extensively documented [8–12] than the effects on the fauna [13–15]. The knowledge of the effects of fire on insect herbivores and their natural enemies is even more limited [3, 16, 17].

The general literature on the responses of insects to fire in comparison with the responses to other forms of management in open habitats indicates that a significant decrease of insects occurs soon after a fire. The magnitude of the decrease is related to the degree of exposure to flames and to the mobility of the insect [18]. In cerrado, a very rapid and vigorous regrowth of vegetation occurs [19] and this regrowth may favor an increase in the abundance of herbivores. The caterpillar community in the cerrado is species rich and the abundance of most species is low but is highly variable throughout the year [20, 21], due primarily to the climate variability that characterizes the two seasons (dry and wet) in the region. This pattern has also been observed for herbivorous insects in New Guinea. It is characteristic of the herbivorous insect communities in general and is also typical of tropical regions [22]. Among the mechanisms that influence these herbivorous insect community patterns, fires, a very frequent historical phenomenon in the cerrado, appear to be an important modifying influence on lepidopteran communities.

The objective of this study was to compare the richness, relative abundance, frequency, and species composition of caterpillars between two cerrado areas, one recently burned and one unburned since 1994. The study hypotheses that the richness, relative abundance, frequency, and species composition of the caterpillars on the host plants vary between recently burned areas and areas without recent burning (used as a control). We predict that the abundance and species richness of caterpillars will increase significantly in a recently burned area as a result of the intense regrowth of vegetation in the postfire environment [19]. The postfire environment differs greatly from the prefire environment because of the higher phenological synchrony of plants and because of changes in microclimate result from to increased exposure to the sun.

2. Methodology

External folivorous caterpillars were surveyed on two plant species, *Erythroxylum deciduum* A. St.-Hil. and *E. tortuosum* Mart. (Erythroxylaceae), in two adjacent areas of cerrado *sensu stricto*, on the experimental farm “Fazenda Água Limpa” (FAL) (15°55′S and 47°55′W), DF, Brazil. Both plant species were abundant and had similar size in the burned and unburned areas. This system, including only two plant species in the genus and their caterpillars, was chosen for study due to the need for simplification in the analysis and reduction of variables. This choice also reflected the ease of collection and identification and the prior knowledge of the system in the protected areas of the cerrado. The two plant species occur at high densities in the cerrado region and their lepidopteran fauna is known from previous studies in unburned areas [20, 23]. An accidental fire affected the entire area in 1994, and the area suffered another accidental fire in August 31, 2005. The area burned in 1994 was viewed as a control, and the area burned in 2005 was considered recently burned. Data were collected from September 2005 through August 2006.

In both study areas (recently burned and control), external folivorous caterpillars were collected weekly from foliage of 50 individuals of each of the two species of plants. All caterpillars were collected, photographed, numbered as morphospecies, and individually reared in the laboratory in plastic pots (except for gregarious caterpillars), with leaves of the host plant as a food. The adults obtained from laboratory rearing were, as far as possible, identified and deposited in the Entomological Collection, Departamento de Zoologia, Universidade de Brasilia.

A binomial test of two proportions was applied with a significance level of 0.05 to evaluate the occurrence of a consistent difference in the proportion of plants with caterpillars (relative abundance and species richness) between the areas [24]. Species rarefaction curves were constructed to analyze the species richness of caterpillars in each area [25]. EcoSim 7.0 software was used to construct these curves based on 1000 replications [26].

Table 1: Number of plants with caterpillars, abundance, and richness of caterpillars on two species of *Erythroxylum,* in two areas of cerrado *sensu stricto* in the FAL (burned and control areas) from September 2005 to August 2006.

Variables	Areas		Total
	Control (%)	Burned (%)	
Inspected plants	2,065 (49.2)	2,131 (50.8)	4,196
Plants with caterpillars	226 (10.9)	333 (15.6)	559
Abundance of caterpillars	346 (35.6)	626 (64.4)	972
Richness of caterpillars	29 (59.0)	36 (74.0)	47*

*Species richness is not the sum total of the richness of the two areas because some species occur in both areas.

The Shannon-Wiener index (H'), Simpson index (D) and Berger-Parker index (D_{bp}) were used to compare the diversity and dominance of the community of caterpillars on *Erythroxylum* in the two study areas. The indices were obtained with DivEs 2.0 software [27]. The Jaccard similarity index was also applied to evaluate the degree of similarity of the species composition of two communities. If the Jaccard index is equal to one ($B = 0$ and $C = 0$), all species are shared between the two communities. If the Jaccard index is near 0, few if any species are shared.

3. Results

We inspected a total of 4,196 plants, with similar numbers in both areas (Table 1). A total of 972 caterpillars were found on 13.3% of the plants inspected. The number of plants with caterpillars (frequency) differed significantly between areas ($p1 = 0.11$; $p2 = 0.16$; $Z = -4.46$; $P < 0.001$). The probability of finding a plant with a caterpillar in the control area (one out of nine plants inspected) was smaller than in the burned area (one to six plants). The relative abundance of caterpillars also differed significantly ($p1 = 0.17$, $p2 = 0.30$, $Z = -9.69$, $P < 0.001$) between areas. Almost twice as many caterpillars were found in the burned area as found in the control area (Table 1).

Forty-seven species or morphospecies (hereafter treated as species) of caterpillars were recorded, belonging to at least 15 families (two species belonged to unidentified families). The burned area had 36 species, compared with 29 species in the control area (Table 1). However, this difference in species richness between the areas was not significant ($p1 = 0.08$; $p2 = 0.06$; $Z = 1.57$; $P > 0.05$). Even after adjustment by the rarefaction method to a common basis of an equal number of caterpillars in both areas ($n = 346$) the species richness did not differ, and the estimated number of species varied between 24 and 32 (Table 2; Figure 1).

The value of dominance was higher in the burned area (34.5%) than in the control area (29.8%) (Table 2). Likewise, the dominance for the burned area, estimated by rarefaction, was between 31.2% and 37.9%, significantly higher than the value estimated for the control area on a common basis of 346 caterpillars in both areas (Table 2). These results are

TABLE 2: Diversity of caterpillars on two species of *Erythroxylum* in two areas of cerrado *sensu stricto* in the FAL (recently burned and control) from September 2005 to August 2006: number of caterpillars, species richness, estimated species richness through rarefaction in the control area (n = 346, 95% confidence interval), dominant species and dominance observed in both areas, estimated dominance by rarefaction in the control area (n = 346, 95% confidence interval), diversity index (H'), and dominance (D and D_{bp}).

	Control area	Burned area
Number of caterpillars	346	626
Observed species richness	29	36
Estimated richness (Rarefaction, n = 346)	—	24–32
Dominant species	*Antaeotricha sp.*	*Antaeotricha sp.*
Observed dominance	29.8%	34.5%
Expected dominance	—	31.2– 37.9%
Diversity of Shanon-Wiener (H')	1.01	0.89
Dominance of Simpson (D)	0.16	0.21
Dominance of Berger-Parker (D_{bp})	0.30	0.35

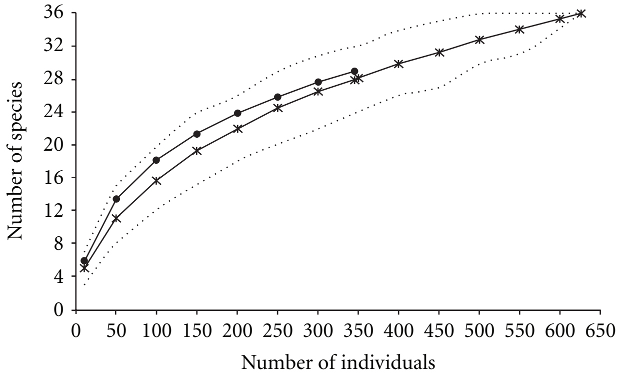

FIGURE 1: Rarefaction curves of caterpillar species of the control area (line with circle) and the burned area (line with star) in relative to the number of individuals estimated from randomizations of the order of 1000 samples in cerrado *sensu stricto* in the FAL from September 2005 to August 2006. The dotted line indicates 95% confidence intervals.

TABLE 3: Abundance of caterpillars and Jaccard similarity index between the two areas of cerrado *sensu stricto* in the FAL (recently burned and control) from September 2005 to August 2006 based on caterpillars found on two species of *Erythroxylum*.

Months	Abundance		Jaccard index
	Control area	Burned area	
Sep	16	0	0.00
Oct	12	17	0.22
Nov	3	7	0.20
Dec	12	30	0.29
Jan	31	40	0.70
Feb	16	27	0.50
Mar	12	33	0.27
Apr	26	42	0.25
May	132	242	0.33
Jun	51	144	0.62
Jul	26	37	0.29
Aug	9	7	0.00
Total	346	626	0.38

also consistent with the dominance index values D and D_{bp}, which were higher in the burned area. The diversity index H' was higher in the control area (Table 2).

An unidentified species of *Antaeotricha* (Elachistidae) was dominant, with 29.7% and 34.5% of the individuals found in the control and burned areas, respectively. Ten species recorded in the control area showed intermediate dominance, between 1.2 and 7.5%, whereas six species showed intermediate dominance in the burned area, with values between 1.1 and 8.0%. The proportion of rare species, those represented by less than 1% of all caterpillars, was significantly higher ($p1 = 0.55$, $p2 = 0.75$, $Z = -1.68$, $P < 0.05$) in the burned area ($n = 27$) than in the control area ($n = 16$).

The similarity between the study areas was low (Sj = 0.38), even on a monthly basis, with January (Sj = 0.70) and June (Sj = 0.62) being the sole exceptions (Table 3). Of the 47 species recorded, 38.3% (n = 18 species) were common to the two areas (Table 4), and 25.5% of the species (n = 12) were restricted to the control area. The species restricted to the control area included the gregarious moth *Hylesia shuessleri* Strand, 1934 (Saturniidae) and the solitary *Dalcerina tijucana* (Schaus, 1892) (Dalceridae), both dietary generalists (Table 4). Approximately 40% of the species (n = 18) were found only in the burned area. These species included *Fregela semiluna* (Walker, 1854) (Arctiidae), a generalist species, and *Eloria subapicalis* (Walker, 1855) (Noctuidae) a dietary specialist. The effects of the fire appear to be more evident for Limacodidae as five of the eight species of this family found in the survey occurred exclusively in the control area. Certain species, however, appear to benefit from the effects of fire, for example, three species of Noctuidae found exclusively in the burned area: *Cydosia mimica* (Walker, 1866), *Cydosia punctistriga* (Schauss, 1904) and Noctuidae sp. The five most abundant species (more than 15 individuals per area) were found in both areas and are apparently restricted to the *Erythroxylaceae* in the region (Table 4).

No caterpillars were found on species of *Erythroxylum* until one month after the fire (Table 3). However, the relative abundance of caterpillars was higher in the burned area in all of the following months. Until 12 months after the occurrence of the fire, the caterpillar relative abundance in the burned remained higher than the abundance found in the control area (Figure 2). The temporal occupation of the species of *Erythroxylum* by caterpillars resulted in a pattern whose abundance and richness gradually increased with

TABLE 4: Families and species of caterpillars found on two species of *Erythroxylum* in burned and control areas of cerrado in the FAL from September 2005 to August 2006 (NI = no information about diet breadth; polyphagous = feeds on species from two or more families of plants; restricted = feeds only on species of Erythroxylaceae).

Family	Species	Control area	Burned area	Diet breadth
Arctiidae	*Fregela semiluna* (Walker, 1854)	0	4	Polyphagous
	Paracles sp.	6	2	Polyphagous
Dalceridae	*Acraga infusa* (Schauss, 1905)	4	2	Polyphagous
	Acraga sp. 1	0	1	NI
	Acraga sp. 2	0	2	NI
	Dalceridae sp.	0	1	NI
	Dalcerina tijucana (Schauss, 1892)	1	0	Polyphagous
Elachistidae	*Antaeotricha* sp.*	103	216	Restricted
	Timocratica melanocosta (Becker, 1982)	2	3	Polyphagous
Gelechiidae	*Dichomeris* sp. 1	1	10	Restricted
	Dichomeris sp. 2	22	6	Polyphagous
	Dichomeris sp. 3*	26	160	Restricted
	Dichomeris sp. 4	3	8	Polyphagous
	Dichomeris spp. (duas espécies)*	68	84	Restricted
	Gelechiidae sp.*	44	50	Restricted
Geometridae	*Cyclomia mopsaria* (Guenée, 1857)*	16	24	Restricted
	Geometridae sp. 1	3	0	Restricted
	Geometridae sp. 2	0	1	Restricted
	Stenalcidia sp. 1	0	5	NI
	Stenalcidia sp. 2	1	0	Restricted
Limacodidae	*Limacodidae* sp. 1	0	1	Polyphagous
	Limacodidae sp. 2	0	1	NI
	Limacodidae sp. 3	1	0	NI
	Limacodidae sp. 4	2	0	NI
	Limacodidae sp. 5	2	0	NI
	Miresa clarissa (Stoll, 1790)	0	1	Polyphagous
	Platyprosterna perpectinata (Dyar, 1905)	5	0	Polyphagous
	Semyra incisa (Walker, 1855)	2	1	Polyphagous
Megalopigydae	*Megalopyge albicollis* (Schauss, 1900)	0	1	Polyphagous
	Megalopyge braulio Schauss, 1924	0	1	Polyphagous
	Norape sp.	4	3	Polyphagous
	Podalia annulipes (Boisduval, 1833)	0	1	Polyphagous
Noctuidae	*Cydosia mimica* (Walker 1866)	0	1	Restricted
	Cydosia punctistriga (Schauss, 1904)	0	1	NI
	Eloria subapicalis (Walker, 1855)	0	7	Restricted
	Noctuidae sp.	0	1	Restricted
Notodontidae	*Heterocampa* sp.	7	12	Polyphagous
Oecophoridae	*Inga haemataula* (Meyrick, 1911)	6	1	Polyphagous
	Inga phaeocrossa (Meyrick, 1912)	1	0	Polyphagous
Pyralidae	*Carthara abrupta* (Zeller, 1881)	12	3	Polyphagous
Riodinidae	*Emesis* sp.	1	0	Polyphagous
	Hallonympha paucipuncta (Spitz, 1930)	0	1	Polyphagous
Saturniidae	*Hylesia schuessleri Strand*, 1934	1	0	Polyphagous
Tortricidae	*Platynota rostrana* (Walker, 1863)	0	3	Polyphagous
Urodidae	*Urodus* sp.	0	5	Restricted
Unidentified	sp. 1	1	0	NI
	sp. 2	1	1	NI

* Indicates the five commonest species.

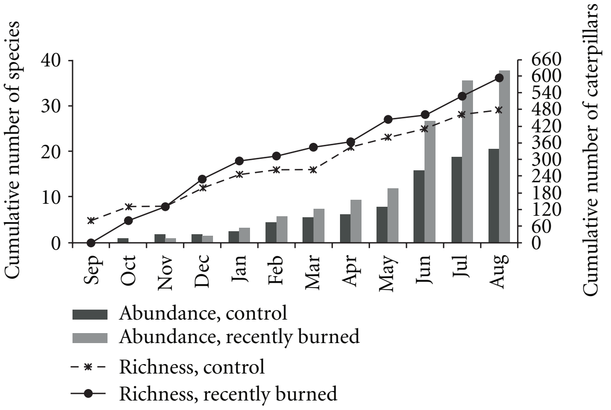

Figure 2: Cumulative number of caterpillars (bars) and species (rows) in two areas of cerrado *sensu stricto* in the FAL (recently burned and control) from September 2005 to August 2006.

sampling effort and showed a greater increase during the dry season, specifically during May and June (Figure 2).

4. Discussion

The sporadic and accidental fires in restricted areas of the cerrado may act to renew the vegetation [19], allowing the reoccupation of sites more rapidly by plant species. Several studies in tropical forests and in the cerrado have shown the importance of sprouting as a mechanism of post-fire regeneration of shrub and tree species [28–32]. The new foliage that results from sprouting attracts a variety of herbivores.

In the cerrado, a low frequency of caterpillars on host plants is a common feature [20, 33–35]. However, recent fire in the cerrado study area produced as 4.7% increase in the frequency of caterpillars on plants of *Erythroxylum*. The reason for this increase may be that fire may benefit herbivores by increasing the availability of resources. This high availability of resources results from the regrowth of plants because many new leaves are synchronously produced.

Although species richness did not differ between areas, the higher dominance observed in the burned area suggests a higher diversity in the control area. The most interesting feature of this system is the increase of rare species in the burned area. This increase may result from intense regrowth, which may produce new oviposition sites and new environments for these species. At the same time, nearby areas were available to act as a source for re-colonization [17]. However, the rarefaction curves did not reach an asymptote. In fact, previous studies [23, 36] indicate that species caterpillars not found in our surveys occur on the two species of *Erythroxylum* that were examined. These additional species include *Erynnis funeralis* (Scudder & Burgess, 1870) (Hesperiidae), *Phobetron hipparchia* (Cramer, [1777]) (Limacodidae) and *Automeris bilinea* Walker, 1855 (Saturniidae). These species are all polyphagous and could be present on other species of host plants.

The variation in the abundance of insects in the cerrado occurs regardless of the passage of fire and remains seasonal [37]. However, the mortality caused by fire produces an immediate reduction in population size. Even, one month after the fire, caterpillars were not found on the plants surveyed. Moreover, the caterpillar abundance on both species of plants during all the subsequent months was higher in the area disturbed by the recent fire. Similar results have been found for adults of certain insect orders, such as Coleoptera, Hemiptera, Hymenoptera and Lepidoptera, in the cerrado of Brasilia [37]. The return to the previous levels of abundance depends on the order to which the insect belongs and ranges from two to more than thirteen months after the occurrence of the fire [3]. Up to 12 months after the occurrence of fire, the abundance of caterpillars associated with the *Erythroxylum* species studied here had not returned to a level comparable with that observed in the control area.

Research conducted in the same region with the community of caterpillars associated with *Byrsonima* (Malpighiaceae), showed that if the fire in the cerrado is recurrent every two years during the dry season, the results are quite different [38] from those previously discussed. In this case, the abundance and species richness of caterpillars in areas with frequent fires were markedly less than the abundance and species richness of caterpillars in areas protected from fire for more than 30 years. These results are consistent with other previous reports that fire reduces the populations of caterpillars [39], and may cause local extinction of some species [40]. However, these results from areas with frequent fires are in contrast to the results found if the fires are accidental and sporadic, as in the case of this study.

Even with smaller losses than those caused by recurrent fires, the recent accidental fire dramatically increased the abundance of caterpillars and as result, the attacks on plants in the postfire period, just at the time at which most synchronous leaf production in the cerrado occurs. For this reason, this process may produce extensive damage to vegetation and may harm biodiversity conservation in the region. Furthermore, a scheme of recurrent burns during several years in the same area results in the biological and physicochemical degradation of the soil and thus in the reduction of aerial biomass [41].

Although we did not replicate each treatment, our results reflect the effect of fire, as we have followed the changes in communities of caterpillars on various plant species for several years in protected areas from fire [21, 23, 38, 42], and in addition, we have surveyed caterpillars on other plant species in postfire conditions, with similar results (unpublished data). Furthermore, some studies suggest the impossibility of replication treatments when it comes from natural phenomena occurring on a large scale, as in the case of burning [43]. Thus, the results of this study indicate that the recent accidental fire had the following effects on the external folivorous caterpillars: (a) killed eggs and larvae at first but had a positive effect on the relative abundance of caterpillars up to one year postfire, (b) increased the frequency of caterpillars associated with two *Erythroxylum* species in the cerrado, (c) did not affect the richness of caterpillars on these plants and (d) changed the caterpillar species composition because the effects of the fire promoted increases of rare or opportunistic species.

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Research Article

Interaction between Bees and the Tristylous Flowers of *Oxalis cytisoides* Mart. & Zucc. (Oxalidaceae)

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The interaction of bees with the tristylous flowers of *Oxalis cytisoides* Mart. & Zucc. (Oxalidaceae) was evaluated. The study was conducted in a semideciduous forest at the Fritz Plaumann State Park in Concórdia, Santa Catarina state. Two *Oxalis cytisoides* aggregations were found and the flower visiting bees were observed. The 3 floral morphs were found at the following proportions: 16 long-, 37 mid-, and 34 shortstyled individuals ($n = 87$). Anthesis lasted one day (6:30 AM to 3 PM). No fruit was formed in the autogamy test; thus, pollination was dependent on the visitors. The pollen grain size varied between the stamens and morphs and formed subsets in accordance with the stigma height (long/mid/short). We collected 165 bees from 30 species visiting the flowers. *Hypanthium divaricatum* was the most abundant bee species (34%) and the males were often observed patrolling the flowers in search of females for mating. Analysis of the pollen loads from 34 females showed that 27 carried *O. cytisoides* pollen. The most frequent bees that carried *O. cytisoides* pollen grains on their bodies were considered pollinator agents, responsible for transferring pollen grains among the floral morphs.

1. Introduction

Heterostyly is a rare phenomenon among plants that has been observed in 28 angiosperm families [1]. Heterostylous species have flowers with different morphs in their populations and may be distylous or tristylous. Each plant holds just one type of flowers. Tristyly is a more complex and rare type of heterostyly, which has been reported in seven botanical families, including Oxalidaceae [1–5].

Charles Darwin formulated an explanation for the adaptive function of heterostyly in 1877, which suggested that the anthers and stigma were positioned to promote cross-pollination between the floral morphs. Darwin [6] also reported observations from Fritz Müller in Santa Catarina for the genus *Oxalis*, who found that flowers do not produce seeds at sites with only one floral morph. However, when the three morphs were planted in a garden, many seeds were produced.

The tristyly species comprises three floral morphs that differ in stamen filament height, pistil style position, pollen grain size and self-compatibility systems. Cross-pollination mediated by insect visitors is favored for this type of flowers [2, 6–9]. The three floral morphs in tristyly populations likely represent the maximum number of sexual polymorphisms in plants that promote cross-pollination through pollinator contact geometry [10]. For legitimate cross-pollination plant requires vectors able to transfer pollen from flowers with long, mid, or short-level anthers to flowers with long-, mid-, or short styles, respectively [7, 9, 11]. All other filament-style length combinations result in little or no seed set [12].

The family Oxalidaceae has radial bisexual flowers with five free sepals and five distinct petals that are slightly connate and often convoluted [13]. There are typically ten stamens with connate filaments at the base; the external filaments are shorter than the internal filaments; and nectar is produced at the base of them or in glands that may alternate with the petals. Typically, the flowers have five styles and stigma that are often globular or punctate [14]. Three Oxalidaceae genera have been observed in Brazil; one of them, *Averrhoa*, (which includes starfruit) is native to Asia, but it is often cultivated in Brazil [13]. *Oxalis* is the largest genus in the family, with approximately 800 species; it is cosmopolitan, and Africa and the Americas are the centers for its diversity. There are approximately 114 *Oxalis* species in Brazil [14]. According to Lourteig [13], *Oxalis cytisoides* Mart. & Zucc. is widely distributed from the northeastern (Ceará) to south regions (Rio Grande do Sul) in Brazil and in Argentina. *Oxalis cytisoides* has an erect herbaceous or shrub habit that can grow up to 1 m high. This species is discontinuously distributed almost entirely across the state of Santa Catarina (southern Brazil), and it is characterized as a heliophile or with a preference for diffuse light and selective hygrophytes that often develop in altered areas, including tropical rain forests, mixed ombrophilous forests on the plateau, and the Upper Uruguay River forest. *O. cytisoides* flowers primarily in spring, which lasts until summer [13].

The frequency of the morphs in the populations and compatible system of many American *Oxalis* species were investigated by Mulcahy [15], Ornduff [2], Weller [16, 17], and Weller et al. [18], demonstrating that in some populations morphs are not in equilibrium and also showing the process of losing styled forms. Recently, Turketti [5] presented an extensive work on the expression of the tristyly in the genus *Oxalis* of South Africa, where it was found that most populations (of 58 different species) were at isopletic equilibrium, which means equal representation of style length morphs. The same thesis [5] studied a special case of two species of *Oxalis* section sagittate which have a different arrange and orientation of the anthers and stigma compared to other *Oxalis*. In these species self-compatibility was more expressive, but the dependence on the pollinator still needs to be tested.

The objective of this work was to study the interactions between the visiting bees and the tristyly flowers of *Oxalis cytisoides*, evaluating the pollination in natural conditions. Our questions were as follows: are there seeds that have been produced in natural conditions? If yes, which flower visitors are potentially responsible for the legitimate transference among the morphs? Additionally, the pollen grains of the different morphs were described.

2. Material and Methods

The study was conducted in the Concórdia municipality between September 2008 and April 2009 at the Fritz Plaumann State Park, 27°16′18′′S, 27°18′57′′S, 52°04′15′′W and 52°10′20′′W, which is predominantly composed of a semideciduous forest. In the study area, we observed two *Oxalis cytisoides* aggregations with clustered distribution of individuals. These aggregations were separated by approximately 1000 m, with sparsely individuals occurring along them. The plants in these aggregations were evaluated together. The three floral morphs of *O. cytisoides* were observed. The morphs did not exhibit remarkable structural differences except for the relative stamen positions, which had different filament and style heights, as schematized in Figure 1.

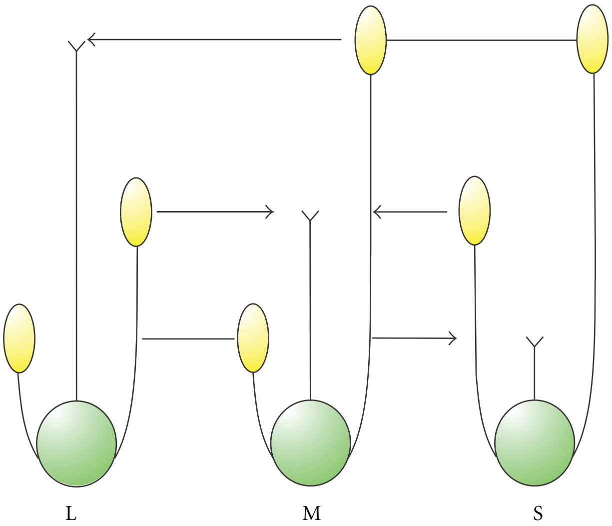


FIGURE 1: Scheme of *Oxalis cytisoides* Zucc flower morphology. L: long-styled flower with medium and short filaments. M: mid-styled flower with short and long filaments. S: short-styled flower with medium and long filaments. The arrows indicate the legitimate cross-pollination, according to the system of the heretostyly plants.

A self-fertilization treatment was used to evaluate *Oxalis cytisoides* self-pollination. In this treatment, 191 preanthesis flower buds from the three floral types were protected with bags made from voile fabric. This sample included 76 long-styled (L) flowers, 63 mid-styled (M), and 52 short-styled (S) flowers. The open flowers and fruits were removed from the branches when the flower buds were bagged. These buds were followed for 1-2 months to assess fruit formation. Pollination in a natural condition was analyzed through examining the fruit formation with seeds. At least 20 fruits of each floral morph were evaluated from each aggregation.

During the flowering period for *O. cytisoides* (September to April), we collected bees visiting the flowers once a month for two consecutive days for a total of 96 hours of sampling; the three flowers morphs were observed equally in time. The collected bees were killed and identified. In addition to collection, we observed the behavior of the bees at the flowers from the three morphs for 30 hours to assess the contribution of bees to *O. cytisoides* pollination. The bee specimens were deposited in the Entomological Collection Paulo Nogueira Neto (CEPANN) of the Bee Laboratory at the Institute of Biosciences in the University of São Paulo.

There are morphological differences in pollen grains from anthers of different filament sizes in *Oxalis* flowers [19]. Based on this information, the *O. cytisoides* pollen grains were analyzed separately to investigate the pollen morphology in the three morphs, samples with stamen anthers that had medium (m) and long filaments (l) in S flowers, short (s) and long (l) filaments in M flowers, and short (s) and mid (m) filaments in L flowers. Pollen grains were removed from the anthers of the three stamen types


(a) (b) (c) (d) (e)

Figure 2: *Oxalis cytisoides*. (a) Plant habit. (b) Flower and buds. (c)–(e): Stamens and pistils from L (c), M (d), and S (e) morphs. Photo scales (c)–(e) = 5 mm.

Table 1: Means (±standard deviation) for pollen grain sizes from the three *Oxalis cytisoides* floral morphs at the equatorial and polar perspectives.

Morph	Filaments	Equatorial	Polar	*P/E*
M	s	24.52 (±1.00)	32.49 (±1.36)	1.32 (±0.06)
L	s	23.89 (±1.17)	33.30 (±1.98)	1.39 (±0.06)
S	m	27.59 (±1.42)	37.30 (±1.65)	1.35 (±0.06)
L	m	26.51 (±0.90)	37.90 (±1.10)	1.43 (±0.05)
S	l	28.89 (±1.13)	40.63 (±1.96)	1.41 (±0.09)
M	l	27.74 (±1.28)	40.59 (±2.67)	1.46 (±0.06)

S: short, M: medium, and L: long.

with different size filaments; they were described separately, and the grain size, and shape were considered. The pollen grains were acetolyzed following the method proposed by Erdtman [20] and subsequently mounted on slides following Barth [21]. For each sample, twenty pollen grains were measured along the polar and equatorial axes in accordance with Silva et al. [22]. A single factor analysis of variance (ANOVA) was used to evaluate the difference in size between the pollen grains [23]. The size of pollen grains is given by the measure from the longest axis in the equatorial view. The data on the pollen grain size were analyzed using the median from boxplots, which were plotted using the program R [24], graphics package version 2.13.0.

Samples of the pollen load from the scopae of the visiting female bees on the *O. cytisoides* flowers were collected for analysis. The pollen grains were subjected to the acetolysis process described above, and they were qualitatively analyzed for the presence or absence of *O. cytisoides* pollen.

This work was performed with the authorization (number 13486-2) for collection and transportation of biological material by IBAMA/SISBIO (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis/Sistema de Autorização e Informação em Bioversidade).

3. Results

A total of 87 flowering individuals were observed: 42 at the first plant aggregation (11 long-, 13 mid-, and 18 short-styled morphs) and 45 at the second aggregation (5 long-, 24 mid-, and 16 short-styled morphs) (Figure 2). Anthesis began at dawn (6:30), and flower abscission began at approximately 15:00.

In self-fertilization test with the three morphs, no fruit was formed in the 191 buds analyzed. In contrast, all those fruits sampled in natural conditions presented seeds.

The pollen grains from *O. cytisoides* (Figure 3) are monads, small and medium size, radial, isopolar, subtriangular in area, subprolate to prolate in shape (Table 1), and tricolpate and have colpate furrows, lolongate endoapertures, and reticulated exine.

The pollen grain size varied between the stamens and morphs (Table 1). Significant differences were observed for

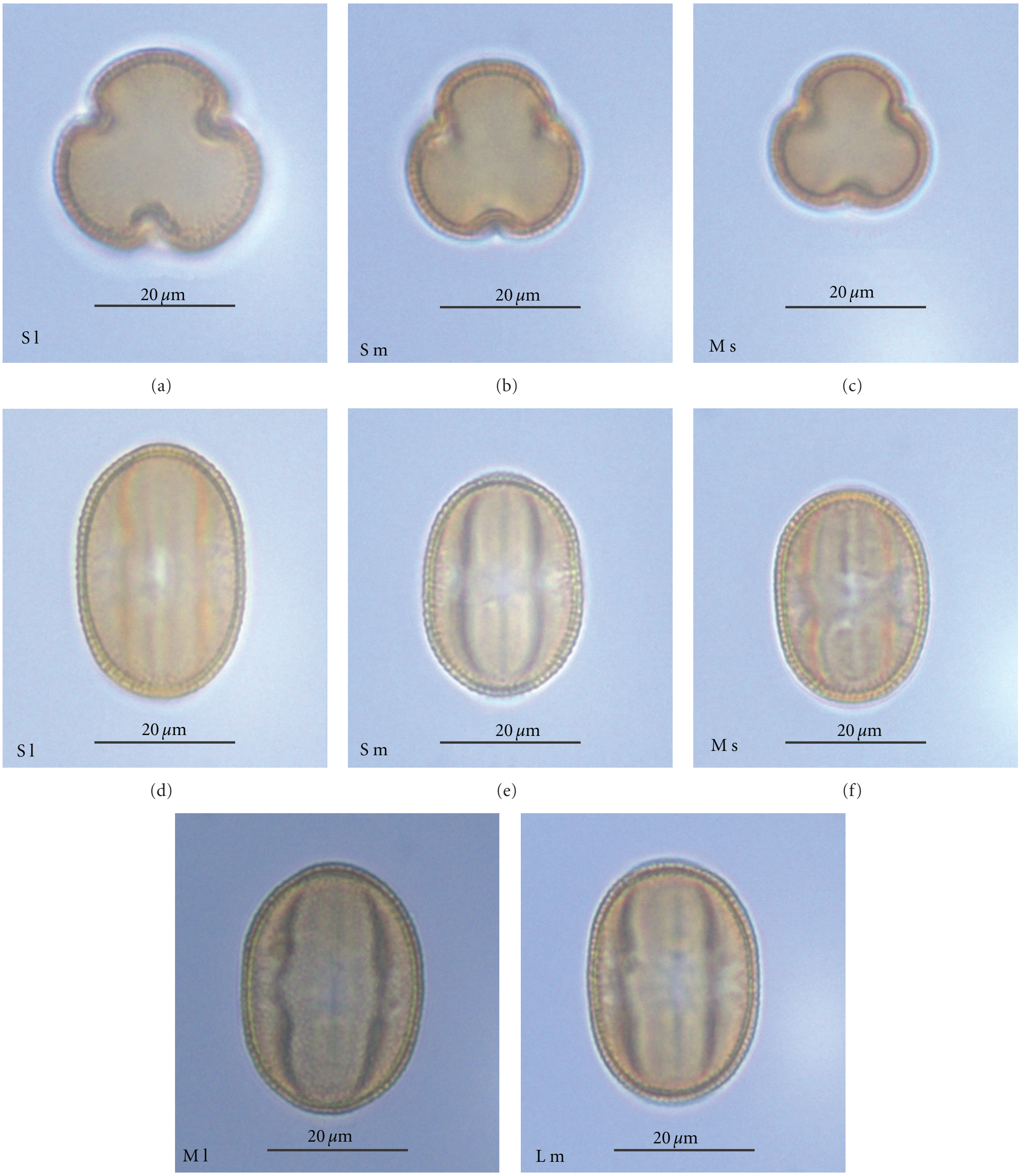


FIGURE 3: Pollen grains from *Oxalis cytisoides*. ((a)–(c)) Polar perspective for pollen grains from long (a), mid (b), and short (c) anthers. ((d)–(i)) Equatorial perspective for pollen grains in long ((d) and (g)), mid ((e) and (h)) and short ((f) and (i)) anthers. Capital letters are the floral morph (style height) and lower letters are the anthers from the pollen grain origin, for example: S l = pollen came from the long filaments (l) of the short styled morph (S).

the pollen grain sizes in polar view from the six samples (mean square, 3.4609; degrees of freedom, 114.00, $P < 0.05$). We also observed that the pollen from short-, mid-, and long-stamen anthers was grouped (Figure 4), forming subsets in accordance with the stigma height.

3.1. Floral Visitors. A total of 165 individuals were sampled and distributed over 30 species and four bee families (Table 2). The visitors were more abundant in December and February, although the number of flowering plants was practically the same throughout the flowering months.

Table 2: Visiting bees of *Oxalis cytisoides* flowers in Concordia, Santa Catarina, southern Brazil.

Family	Species	Ind.	Month
Andrenidae	*Anthrenoides meridionalis* (Schrottky, 1906)*	2 (2 F)	Nov
	Psaenythia bergii Holmberg, 1884*	2 (2 F)	Nov
Apidae	*Ceratina* (*Calloceratina*) sp. 2	1 (1 F)	Dec
	Ceratina (*Crewella*) sp. 12	1 (1 F)	Sep
	Ceratina (*Crewella*) sp. 16*	15 (12 F, 3 M)	Feb-Mar, Sep–Dec
	Lophopedia nigrispinis (Vachal, 1909)*	23 (16 F, 7 M)	Feb, Sep, Nov-Dec
	Odyneropsis sp.	1 (1 F)	Dec
	Paratetrapedia (*Paratetrapedia*) sp. 1	5 (1 F, 4 M)	Feb
	Paratetrapedia (*Paratetrapedia*) sp. 2*	7 (6 F, 1 M)	Nov–Mar
	Paratetrapedia (*Paratetrapedia*) sp. 4	6 (6 M)	Feb, Nov
	Tetrapedia diversipes Klug, 1810*	13 (13 F)	Feb-Mar, Nov-Dec
	Trigona spinipes (Fabricius, 1793)	1 (1 F)	Sep
Halictidae	*Augochlora* (*Augochlora*) sp. 1†	1 (1 F)	Nov
	Augochlora (*Augochlora*) sp. 3	1 (1 F)	Oct
	Augochlora (*Augochlora*) sp. 4	1 (1 M)	Dec
	Augochlora (*Augochlora*) sp. 6	1 (1 F)	Dec
	Augochlora (*Oxystoglossela*) sp. 4	1 (1 F)	Feb
	Augochlorella sp. 2	7 (7 F)	Feb, Nov-Dec
	Augochlorella sp. 5	5 (4 F, 1 M)	Oct–Jan
	Augochloropsis sp. 1	1 (1 F)	Jan
	Augochloropsis sp. 2	1 (1 F)	Dec
	Augochloropsis sp. 12	1 (1 F)	Feb
	Neocorynura sp.	1 (1 F)	Dec
Megachilidae	*Anthidulum mourei* Urban, 1993	1 (1 M)	Nov
	Hypanthidium divaricatum (Smith, 1854)*	57 (17 F, 40 M)	Nov–Apr
	Hypanthidium obscurius Schrottky, 1908†	3 (1 F, 2 M)	Nov–Jan
	Megachile (*Leptorachina*) sp. 1	2 (2 M)	Jan, Mar
	Megachile (*Austromegachile*) *susurrans* Haliday, 1836	2 (2 F)	Dec
	Moureanthidium paranaense Urban, 1995	1 (1 M)	Nov
	Moureanthidium subarenarium (Schwarz, 1933)	1 (1 M)	Nov
Total		165	Sep–Apr

Ind.: number of individuals, month: collection month, * *O. cytisoides* pollen grains in the scopae pollen load, and † number of *O. cytisoides* pollen grains in the scopae. F: female and M: male.

In general the bees visited more than one flower on the same plant and different plants in the same aggregation with no observed preference for a particular morph. *Lophopedia nigrispinis* (Vachal, 1909) (Apidae), *Tetrapedia diversipes* Klug, 1810 and *Ceratina* sp. remained for a few seconds in the flowers and visited between 2 and 5 flowers, preferentially collecting nectar. *Hypanthidium divaricatum* (Smith, 1854) (Megachilidae) and *Ceratina* sp. (Apidae) actively collected pollen and nectar.

Among the sampled bees, the most abundant in the flowers were *H. divaricatum* (Figures 5(a) and 6(a)–6(c)) and *L. nigrispinis* (Figures 6(d)–6(f)), which corresponded to 34% and 14% individuals, respectively. Pollen grains were often observed adhered to the mouthparts of the sampled bees (Figures 6(c) and 6(f)). *H. divaricatum* males were frequently observed patrolling *O. cytisoides* flowers searching for females, and a mate was recorded on certain occasions (Figure 5(b)).

Oxalis cytisoides pollen was observed in 25 samples of pollen material removed from the scopae of 32 females that belonged to nine bee species, which indicates that these females effectively collect this floral resource (Table 2).

4. Discussion

The three floral morphs of *Oxalis cytisoides* were clearly distinguished in the studied area. The self-fertilization (bagged flowers) tests showed no fruit production, even in the mid- and short-styled flowers, which would be easily contaminated with pollen from the above anthers. On the other hand flowers left under natural conditions produced fruits, which demonstrated participation by pollinators. In the studied

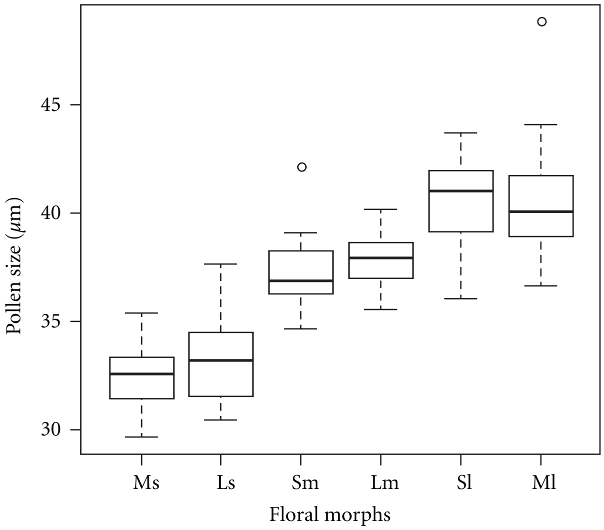
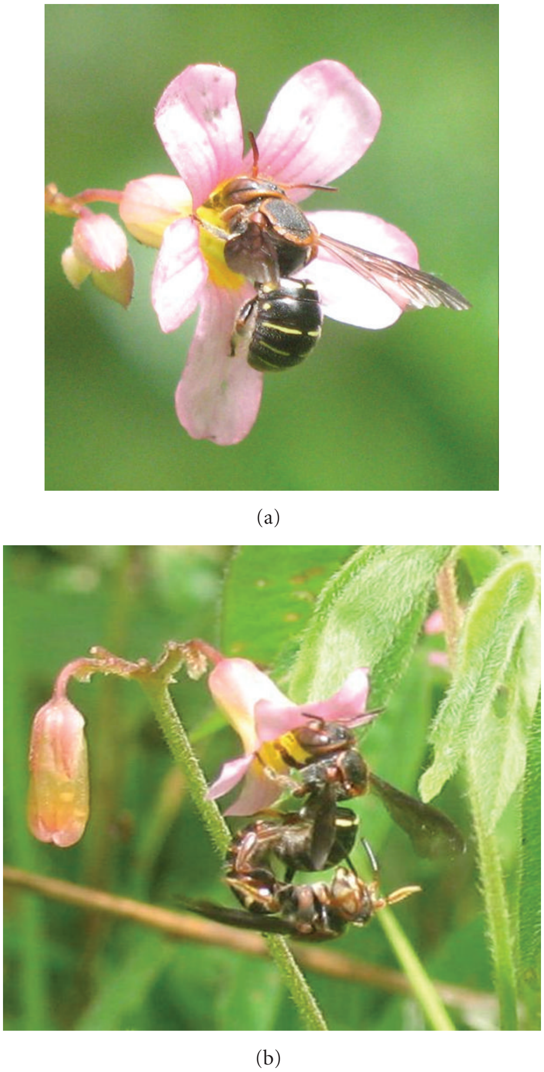


Figure 4: Box plot of pollen grain sizes for the three flower morphs measured from the polar perspective. Box plots show the medians as well as 1° and 2° quartiles and ranges; the circles indicate extreme values (outliers).


(a)

(b)

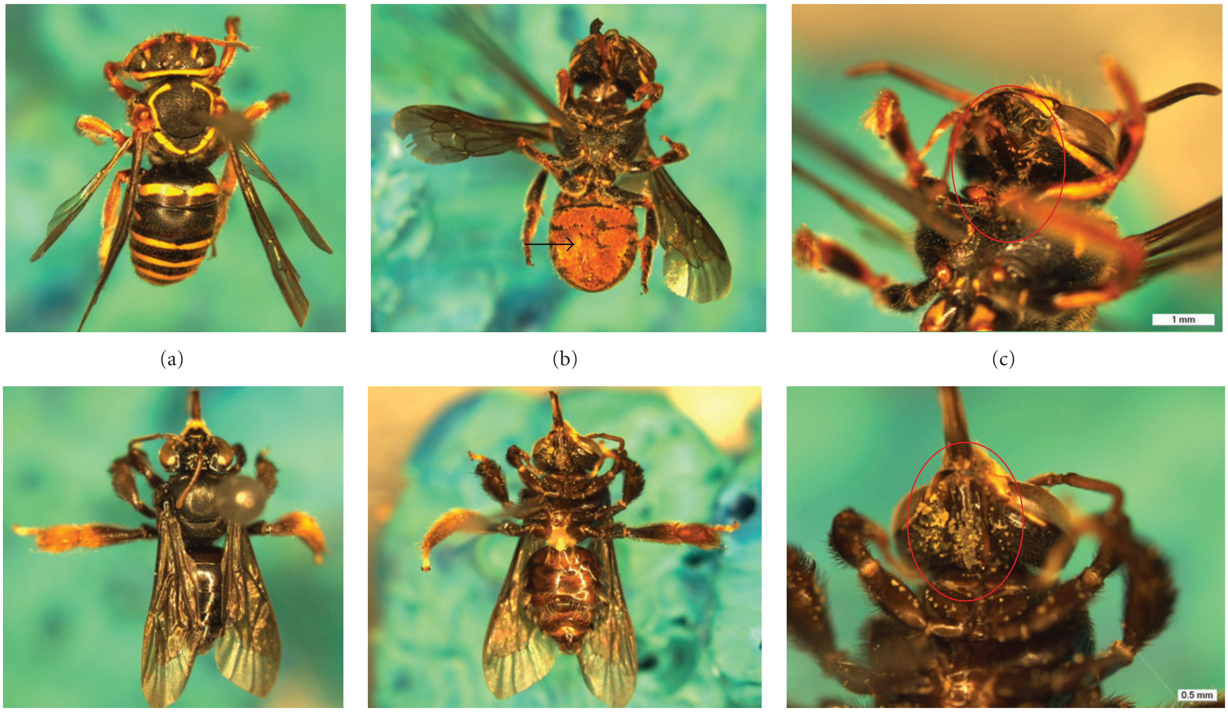
Figure 5: *Hypanthidium divaricatum* visiting (a) and mating (b) at *Oxalis cytisoides* flowers.

area we sampled 30 bee species visiting the flowers of *O. cytisoides*. Among them there were some very frequent in the visits and that were flying 4–6 months during the whole flowering period of the plant species, like *Hypanthidium divaricatum*, *Lophopedia nigrispinis*, and *Ceratina* sp. 16. These three species are potential candidates for pollinators, due to the frequency and abundance in the flowering period, and also because the females collected actively pollen from *Oxalis* flowers (demonstrated by behavior and the analysis of the pollen load of the scopae). Pollen collection requires more time and ability at the flowers during the visits. This increases the permanence time at the flower and contact with the reproductive parts, assisting in pollen transference to the stigma. Simultaneously, during the visits, the pollen can adhere to the visitor bodies, like to the forehead, abdomen, and thorax. These pollens adhered to the hairs are likely more important for pollen transfer than the pollens in the scopae, because of the position they will touch into the next flower. Therefore, the bee species with these characteristics were most likely to promote cross-pollination for *O. cytisoides* in the studied area, guarantying the verified fruit set and also the maintenance of the three morphs in the populations. It is known that the trimorphic condition tends to break down the polymorphism and evolves a homostylous condition in populations with a deficit of effective pollinators [4, 12].

Hypanthidium divaricatum shows a special behavior and preference to the flowers of *O. cytisoides*. The males patrol and seek for females to copulate in the plants. This behavior indicates that males recognize this plant as a preferred species of their females, showing specialization. A similar fact was also reported for *Ancyloscelis* bees and plants of the Pontederiaceae family [25] and for *Cephalurgus anomalus* Moure and Oliveira and plants of the Malvaceae family [26, 27]. Further results reinforcing the specialization were attested by the pollen load of the females of *H. divaricatum*, which were carrying most grains of *O. cytisoides*. This bee species was found visiting other plants in Concórdia, but of 86 individuals collected in the region 46 (53%) were visiting *O. cytisoides* flowers [28].

According to our results *H. divaricatum* is flying between November and April covering the most period of flowering of *O. cytisoides*. Since the flower season for this species starts in September, probably the 2 other bee species (*Ceratina* sp. 16 and *L. nigrispinis*) with high frequency in the flowers may act as effective pollinators at the beginning of the season.

Luo et al. [29] studied the South American species *Oxalis debilis* Kunth, which was introduced in China and found that it does not predominantly reproduce vegetatively, as was previously assumed for this species. The pollination of *O. debilis* was performed by bees that collected nectar (*Apis cerana* Fabricius, 1793) as well as pollen (*Ceratina* (*Pithitis*) *smaragdula* Fabricius, 1787, and *Ceratina* sp.). According to Björkman [30], disc-shaped and small flowers allow for



(a) (b) (c)

(d) (e) (f)

FIGURE 6: (a)–(c): *Hypanthidium divaricatum* ((a): male dorsal view, (b): female ventral view with loaded abdominal scopae (arrow) and (c): close-up of the male mouthparts with pollen grains attached to the hairs). (d)–(f): Male *Lophopedia nigrispinis* (d: dorsal view, e: ventral view and f: close-up of mouthparts with pollen grains attached to the hairs).

nectar access by several species, which facilitates the cross-fertilization of the plant. This is probably the case of *O. cytisoides* studied herein which received a spectrum of 30 bee species, including short and long tongued bees. But, it is important to emphasize that to the plant visitors with high frequency and carrying pollen grains are likely more effective for the cross-pollination than those sporadically visitors. However, future studies on stigmatic receptivity and specificity in *O. cytisoides* are necessary for a more accurate conclusion about the role of each visitor.

The morphology and ornamentation of the pollen grains from *O. cytisoides* are consistent with the description for nine species in the *Oxalis* genus by Rosenfeldt and Galati [19]. The largest pollen grains were produced in the high level anthers (long stamens), intermediate size pollen was produced by mid-level anthers, and smaller grains were produced by low level anthers, as has been reported for other *Oxalis* species [14, 19, 31] and would be expected in a tristyly species. At the load of the bees it was not possible to distinguish or quantify precisely the anther origin of the pollen grains, because of some overlap in their size. But the three types of pollen were presented in the samples of the seven bee species carrying *Oxalis* pollen (Table 2). We believe that female bees are able to collect the pollen grains from all the anther levels without a problem, since the flowers of *Oxalis* do not have special morphology to hide the resource, like it is known for the tristyly *Eichhornia* species [9, 12].

Compatibility tests between morphs of species of the genus *Oxalis* studied by Ornduff [2] and Pacheco and Coleman [31] revealed that legitimate pollination (following heterostyly scheme) is more successful than illegitimate pollination for frequency and seed production. Although we did not conduct cross-pollination tests, we found that under natural conditions fruits were produced, demonstrating the importance of the local visiting bees as agents for the correct pollen transference.

Finally, to the bee perspective, it is necessary to highlight that *O. cytisoides* seems to be an important source of floral rewards for the local bee fauna. Certain studies refer to *Oxalis* as a flower resource for honeybees [32, 33]. But at the study area we verified that it deals with a relevant source of nectar and pollen for many native bee species, especially solitary bees. According to Krug [28], about 27% of the known *Apiformes* species occurring in the Fritz Plaumann Park in Concordia were visiting the flowers of *O. cytisoides*.

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Research Article

Flower-Visiting Social Wasps and Plants Interaction: Network Pattern and Environmental Complexity

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Network analysis as a tool for ecological interactions studies has been widely used since last decade. However, there are few studies on the factors that shape network patterns in communities. In this sense, we compared the topological properties of the interaction network between flower-visiting social wasps and plants in two distinct phytophysiognomies in a Brazilian savanna (Riparian Forest and Rocky Grassland). Results showed that the landscapes differed in species richness and composition, and also the interaction networks between wasps and plants had different patterns. The network was more complex in the Riparian Forest, with a larger number of species and individuals and a greater amount of connections between them. The network specialization degree was more generalist in the Riparian Forest than in the Rocky Grassland. This result was corroborated by means of the nestedness index. In both networks was found asymmetry, with a large number of wasps per plant species. In general aspects, most wasps had low niche amplitude, visiting from one to three plant species. Our results suggest that differences in structural complexity of the environment directly influence the structure of the interaction network between flower-visiting social wasps and plants.

1. Introduction

Wasps are a significant portion of the flower-visiting guild that may overlap bees in nectar exploitation in several ecosystems [1, 2]. Although wasps are more frequently regarded as floral resource thieves [3–6], recent papers show that wasps can also effectively contribute to pollination [7]. In a study with *Schinus terebinthifolius* Raddi (Anacardiaceae), Sühs et al. [8] showed that social wasps, especially *Polistes versicolor* (Olivier, 1791), *Polybia sericea* (Olivier, 1791), *Polistes simillimus* (Zikán, 1951), and *Polybia ignobilis* (Haliday, 1836), were more representative in richness and abundance than bee species, being considered efficient pollinators. Other studies, such as Barros [9] and Hermes and Köhler [10] also demonstrated the efficiency of the wasps as pollinators. Thus, social wasp-plant interaction networks can be considered nonobligatory mutualistic associations.

Interaction networks between plants and their floral visitors are currently among the most widely studied interactions (see review by [11]). The structure of these networks, almost always characterized by mutualistic interactions (pollinators and plants), exhibits a common pattern of nestedness and asymmetry [6, 12–14] with varied specialization degree [15, 16]. According to Blüthgen et al. [15], pollinator webs were highly asymmetric, involving a much higher number of pollinators (usually insects) than plant species. Consequently, pollinators are significantly less specialized on plants than plants on pollinators. On the other hand, the nested pattern common in mutualistic networks means that species with few links interact with a subset of interactive partners with high connectivity [12]. In those webs, the species with many links (hubs species) are the most generalists and give support to network pattern according to Bastolla et al. [17]. Thus, the properties of interaction networks of plant pollinator tend to generalization, even though they are on average more specialized than networks of other mutualisms, such as seed dispersal and the use of extrafloral nectaries [14].

Recent network analyses revealed that plant-pollinator interactions display an intermediate level of complementary specialization [16]. According to Blüthgen and Klein [18], specialization and complementarity are related: complementarity requires a certain degree of specialization of each species, while high generalization is associated with high niche overlap and thus redundancy. Therefore, plant-pollinator interactions exhibit an intermediate structure between generalization and specialization (represented by nestedness, asymmetry, and intermediate level of complementary specialization). These proprieties make these interaction networks robust and more stable to species extinction [17, 19–21].

Studies on the structure of interaction networks between plants and flower-visiting social wasps are scarce [6, 22], as well as knowledge about the diversity of wasps in various environments [1, 10, 23]. In Brazil, studies on the sources of floral resources used by social wasps were carried out in Cerrado (the Brazilian Savanna) [24–26], in an Araucaria Forest [10], in urban areas [27], and in Brazilian Caatinga (a semiarid scrub forest) [28]. Furthermore, there are only two studies dealing with the interaction network between plants and flower-visiting social wasps in Brazil [6, 22], and these studies show only the general pattern of interactions or compare these patterns with networks involving other groups of floral visitors.

Most studies concerning ecological interaction networks relate the network structural pattern with the type of relationship established between the groups of organisms, without taking into account the characteristics of the environment in which the interaction takes place (e.g., [16, 29]). Thus, it becomes difficult to understand the actual biological importance of the interactions. However, interactions are known to be strongly variable in time and space [30–32]. It is known that more structurally complex environments (represented for greater availability of food resources and nesting sites) have greater richness and diversity of species [33, 34], including social wasps [35, 36]. According to Santos [35], vegetation structure influences social wasp communities because it provides support for nesting, food resources, resources for building nests, and foraging area. Thus, hypothetically, environments with different complexities may have interaction networks with different topological properties and, consequently, different specialization degree. Therefore, in this study we propose to know the structure of the interaction network between flower-visiting social wasps and plants in two distinct phytophysiognomies in a Brazilian Savanna area, and to compare them mainly in terms of network structure and its specialization degree.

2. Materials and Methods

2.1. Study Area and Data Sample. We carried out the field collections in the period from November 2007 to October 2008 in two phytophysiognomies (Riparian Forest and Rocky Grassland) of the neotropical savanna reserve of Parque Estadual do Ibitipoca (PEIB) (Ibitipoca State Park), which is localized in Serra de Ibitipoca (Ibitipoca Mountains), MG, Brazil. The area covers 1,488 hectares at coordinates 21°40′44′′S and 43°52′55′′W. According to the Köppen classification, the climate of this region is Cwb (humid mesothermal regime with dry winters and rainy summers). The Riparian Forest exhibits a phytophysiognomic profile of transition from high-altitude savannas to ombrophilous forests, with a physiognomy sequence from shrubby arboreal to predominantly arboreal [37]. This area exhibits a great heterogeneity of plant species mainly due to variations in soil humidity [38]. Furthermore, Fontes [39] highlights the importance of persistence of cloudiness (i.e., high humidity) in this vegetation type. On the other hand, the Rocky Grassland physiognomy exhibits a xeromorphic aspect, with a wide diversity of herbs and shrubs distributed over quartzite outcrops [37]. This area is dominated by plants tolerant to water stress due to the high incidence of light and wind [40].

In each of these two physiognomies, we used one transect of the 800 × 4 m for monthly examinations throughout the twelve months of study. The transects were 1200 m distant from each other for its independence. We carried out the monthly observations in two days between 7:00 and 17:00 h. During this period, we observed for 10 min all the plants that had flowers, and we collected one individual of each one of wasp specie that visited the flowers. This capture was made with an entomological sweep net, according to methods of Aguiar [41]. We identified and stored the plants in the CESJ Herbarium at the Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil (UFJF), with the following access numbers: 51321 to 51347, 52165, and 52166. We identified the wasp species according to Richards [42], and Carpenter and Marques [43], and we stored them at the UFJF entomological scientific collection.

2.2. Data Analysis. To get to know the network connectivity between plants and wasps in the areas of Riparian Forest and Rocky Grassland, we constructed two adjacency matrices (wasp in plant) per area: "quantitative matrix" considering frequency of interactions of each wasp species on each plant species and "qualitative matrix" with data of presence/absence of the wasp species in plant species. The metrics that we used to check the properties of interaction networks were connectance, asymmetry, and nestedness index ($NODF$) using qualitative matrixes, and degree of complementary specialization (H_2') using quantitative matrixes.

We calculated the connectance (C) according to Jordano [44], where $C = I/(F * P)$, "I" is the total number of interactions observed, "F" is the number of wasp species, and "P" is the number of plant species. Percent values of C were obtained by multiplying it by 100. We calculated the average degree for plants using the arithmetic means of degrees (= number of interactions in which each species was involved) of all plant species (sensu [45]); the same was carried out for wasps. We also calculated the balance between numbers of plant "I" and wasps "J" species in each network using the following equation: $W = (I - J)/(I + J)$, where "W" is the web asymmetry. Values equal zero for balanced webs, positive numbers indicate more plant species, and negative more wasp species, rescaled to (–1, 1), see [15, 46]. To estimate the nestedness value of networks, we used the *NODF* index: *nestedness metric based on overlap and decreasing fill,* see [47], calculated by the software Aninhado 3.0 [48]. We check the significance of *NODF* with a Monte Carlo procedure with 1,000 randomizations, using null model II, in which the interaction probability between a wasp and a plant is proportional to their total number of interactions. *NODF* index is strongly recommended due to its theoretical and statistical consistency [47].

In order to verify the degrees of networks specialization, we calculated the degree of complementary specialization (H_2') for each network using the quantitative matrixes. This degree is derived from Shannon index, and it is robust to changes in sampling intensity and the number of interacting species in the network. It is based on the deviation from the expected probability distribution of the interactions (see [15, 49]), and its results vary from 0 (extreme generalization) to 1.0 (extreme specialization).

Besides the metrics for network characterization, we calculated the specialization degree (d') of the wasp species for each network. The d' index is a standardized measure of the Kullback-Leibler distance, which measures the specialization of a species based on the frequency of the total number of interactions in the network [49]. This index ranges from 0 to 1.0 indicating extreme generalization and specialization of the species, respectively [49]. We used the R software version 2.13.2 (freeware) to calculate the H_2' and d' indices and to construct the bipartite graph.

We also verify the importance of the plant species on the interaction with the wasps in both areas (Riparian Forest and Rocky Grassland). For this we used the importance index developed by Murray [50]. In the equation $Ij = \Sigma[(Cij/Ti)/S]$, "Ti" is the total number of plant species visited by each wasp species, "S" the total number of visiting species, and "Cij" corresponded to the binary data (0/1) (see also [6]). The values of this index range from 0 to 1.0, where 1.0 is the maximum importance value of each plant species for each wasp species.

In addition, we verify the monthly diversity (*Shannon* index) of social wasps visiting flowers in each area studied using the PAST software (freeware). We compared the monthly values of diversity between areas using the *Mann-*Whitney test(U-test) by the software BioEstat 4.0 (freeware). We also calculated the niche amplitude of social wasps by means of the Shannon index: $H' = -\sum p_k \cdot \ln p_k$, where "$p_k$" is the proportion of the individuals collected in a plant species "k," and "ln" is the Neperian logarithm of the value "p_k."

3. Results

The interaction networks between wasps and plants in the two landscapes showed different patterns (Table 1). The network was more complex in the Riparian Forest, with a larger number of species and individuals and a greater amount of connections between them (see Table 1, Figure 1, and Table 2). The network specialization degree (H_2') was more generalist in the Riparian Forest than in the Rocky Grassland. This result was corroborated by means of the nestedness index (*NODF*), by which only the Riparian Forest presented a significant nestedness value (Table 1). The interactions in the Rocky Grassland tended towards specialization, with relatively high values of H_2' and nonsignificant nestedness. In both networks was found asymmetry, with a large number of wasps per plant species. The Riparian Forest had more wasp species diversity than Rocky Grassland ($U = 105.5$; $P < 0.05$). The composition of the plant community visited by wasps was different in the two phytophysiognomies. Moreover, the most important plant species in the Riparian Forest was also present in the Rocky Grassland, and also exhibiting high importance (Figure 2). Despite these differences, in both areas, the most important source of floral resources for social wasps, presenting the highest richness of visitors, was the species belonging to the Asteraceae family (with nine species). *Baccharis* sp.1 was the species that presented the greatest importance index for the wasp species in both phytophysiognomies (Figure 2). On the other hand, the families with only one visiting individual were Orchidaceae, (*Prosthechea vespa* (Sw.) W. E. Higgins), Lythraceae (*Cuphea* sp1), Fabaceae (*Periandra* sp1), Theaceae (*Gordonia fruticosa* (Schrad.) H. Keng), and Poaceae (*Trachypogon spicatus* (L. F.) Kuntze), with low importance for wasp in the network interactions here studied.

Table 1: Metrics of networks for the wasp-plant interactions studied at Ibitipoca State Park, MG, Brazil, during the period from November 2007 to October 2008.

Network metrics	Riparian forest	Rocky grassland
Number of plant species	18	11
Number of wasp species	15	8
Number of associations	44	14
Degree of plant species (average degree ± SE)	2.44 ± 0.57	1.27 ± 0.14
Degree of wasp species (average degree ± SE)	2.93 ± 0.62	1.75 ± 0.52
Network connectance	16.29%	15.90%
H_2' index	0.347	0.521
Web asymmetry	0.09	0.15
Nestedness value (NODF)	17.71, $P < 0.01$	11.61, $P = 0.51$

Although plant community visited by wasps was different between the two studied physiognomies, the most important plant species (specialists) in the Riparian Forest were also present in the Rocky Grassland. With the exception of two wasp species found only in the Riparian Forest, the landscapes presented the same wasp species. Of all wasp species observed in the Riparian Forest, *Mischocyttarus confusus* Zikán, 1935, was the most generalist, interacting with eight out of the 18 plant species (Figure 3 and Table 2). In Rocky Grassland, the species *Mischocyttarus drewseni* Saussure,

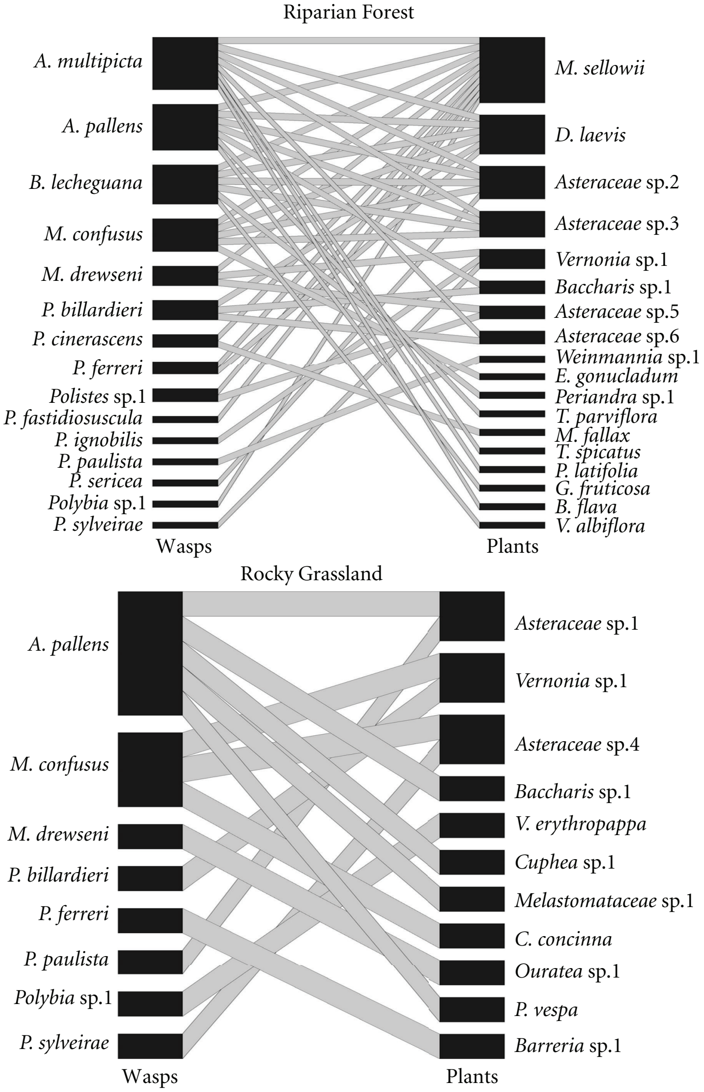

Figure 1: Wasp-plant interaction networks at Ibitipoca State Park, Minas Gerais, Brazil, during the period from November 2007 to October 2008. Riparian Forest: 18 plant species and 15 wasp species, Rocky Grassland: 11 plant species and eight wasp species (see Table 3 for classification and authors of species).

1857, was the species that presented higher level of generalization, interacting with five plant species. Furthermore, *M. confusus* and other five species (*M. drewseni*, *Polybia paulista* (von Ihering, 1896), *Polybia* sp1, *Polistes billardier*i Fabricius, 1804, and *Protonectarina sylveirae* (Saussure, 1854)) had an interaction degree higher than the average found to the group in Riparian Forest, all with degree of 2.93 (see Table 1). These species, with the exception of *P. billardieri*, were found in higher abundance in Riparian Forest. Looking for the wasp species interacting with only one plant, in both vegetation physiognomies we found six species in this condition. This represents 40% of Riparian Forest and 70% of Rocky Grassland species (Table 2).

In general aspects, most of wasps had low niche amplitude, visiting from one to three plant species (Table 2). In Riparian Forest, *M. confusus* and *M. drewseni* were the species with higher *niche amplitude, visiting eight and seven plant species. In Rocky Grassland, the variation in niche amplitude was lower, from zero to 1.561, and M. drewseni* was the wasp with higher *niche amplitude, visiting 11 plant species* (Table 2). Some wasp species present the value zero in amplitude reflecting a very small number of observed interactions with distinct plant species.

4. Discussion

The structural aspects of the environments where interaction networks are studied must be considered in order to fully understand the predominant patterns and processes in ecological networks. The two interaction networks between flower-visiting social wasps and plants evaluated in this study presented remarkable differences directly related to variation in landscape. In general, networks involving floral visitors

Table 2: Abundance, number of plant species visited, and niche amplitude of the species of social wasps collected in Ibitipoca State Park, MG, Brazil, during the period from November 2007 to October 2008. "RF" means Riparian Forest and "RG" means Rocky Grassland. See Table 3 for the classification and authors of species.

Wasp species	Abundance of individuals		No. of plant species visited		Niche amplitude (H')	
	RF	RG	RF	RG	RF	RG
Mischocyttarus confusus	16	1	8	1	1.808	0
Polybia sp. 1	14	7	5	3	1.400	0.955
Polybia paulista	16	2	6	1	1.548	0
Polistes billardieri	4	1	3	1	1.040	0
Mischocyttarus drewseni	11	6	7	5	1.768	1.561
Protonectarina sylveirae	7	1	3	1	0.796	0
Polistes sp. 1	2	0	2	0	0.693	—
Polybia sericea	2	0	2	0	0.693	—
Apoica pallens	2	1	1	1	0	0
Polistes ferreri	2	1	1	1	0	0
Polybia ignobilis	3	0	2	0	0.636	—
Agelaia multipicta	1	0	1	0	0	—
Brachygastra lecheguana	1	0	1	0	0	—
Polistes cinerascens	1	0	1	0	0	—
Polybia fastidiosuscula	1	0	1	0	0	—

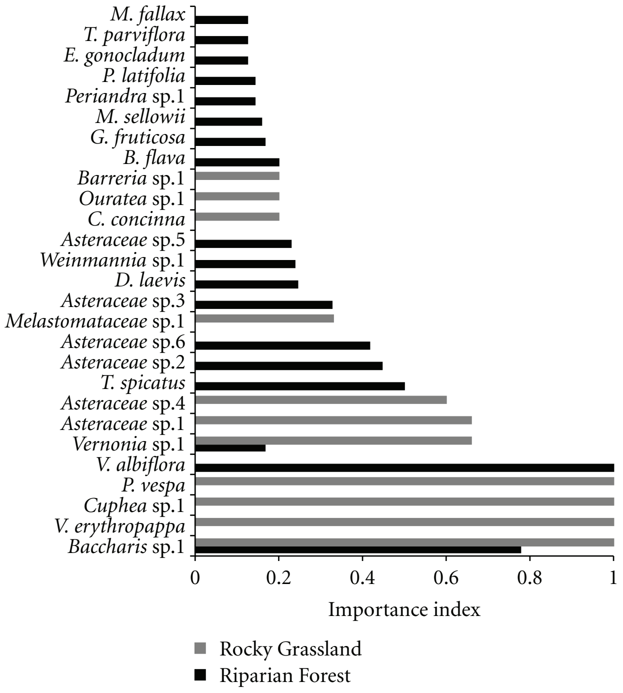

Figure 2: Importance index of plant species in the two areas of Ibitipoca State Park (Riparian Forest and Rocky Grassland), MG, Brazil, during the period from November 2007 to October 2008.

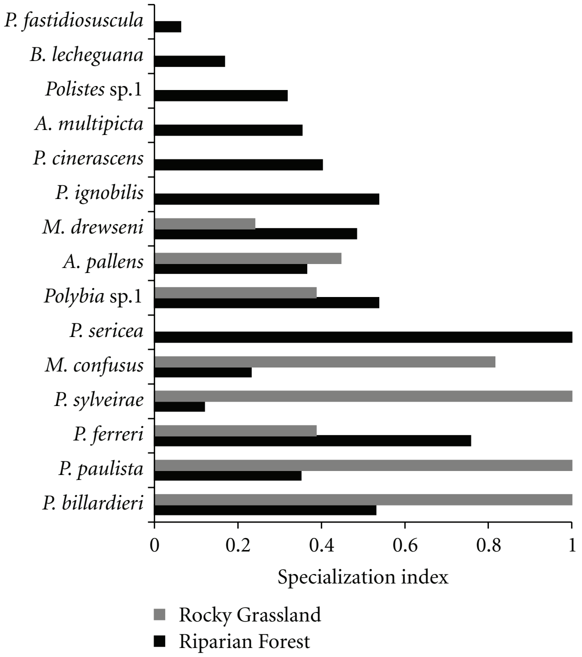

Figure 3: Specialization index of social wasp species in the two areas of Ibitipoca State Park (Riparian Forest and Rocky Grassland), MG, Brazil, during the period from November 2007 to October 2008.

and plants present a nested pattern and intermediate levels of specialization [6, 15, 16, 51]. Nevertheless, we observed two distinct patterns between the areas assessed: a more generalist (significant nestedness and lower specialization degree) and a more specialist pattern (without significant nestedness and with larger specialization degree than 0.5). The species degrees also showed the differences of network patterns (Table 1). Despite these differences, both networks showed asymmetry, with larger number of plant species than wasp species. This result does not corroborate the structure of other pollinator-plant networks, which involve a much higher number of pollinators than plant species [15]. In these networks, pollinators are significantly less specialized on plants than plants on pollinators. Here we found

Table 3: Classification and authors of species.

Family/plants species	Family/tribe/wasps species
Apocynaceae	Vespidae
*Mandevilla sellow*ii (Müll. Arg.) Woodson	Epiponini
Ditassa laevis Mart.	*Agelaia multipicta* (Haliday, 1836)
Asteraceae	*Apoica pallens* (Fabricius, 1804)
sp1	*Brachygastra lecheguana* (Latreille, 1824)
sp2	Mischocyttarini
sp3	*Mischocyttarus confusus* Zikán, 1935
sp4	*Mischocyttarus drewseni* Saussure, 1857
sp5	Polistini
sp6	*Polistes billardieri* Fabricius, 1804
Vernonia sp1	*Polistes cinerascens* Saussure, 1854
Baccharis sp1	*Polistes ferreri* Saussure, 1853
Vanillosmopsis erythropappa (DC.) Sch. Bip.	*Polistes* sp1
Cunoniaceae	Epiponini
Weinmannia sp1	*Polybia fastidiosuscula* Saussure, 1854
Erythroxylaceae	*Polybia ignobilis* (Haliday, 1836)
Erythroxylum gonocladum (C. Martius) O. E. Schulz	*Polybia paulista* (von ihering, 1896)
Fabaceae	*Polybia sericea* (Oliver, 1791)
Periandra sp1	*Polybia* sp1
Lythraceae	*Protonectarina sylveirae* (Saussure, 1854)
Cuphea sp1	
Melastomataceae	
sp1	
Trembleya parviflora (D. Don) Cogn.	
Myrtaceae	
Myrcia fallax (Rich.) DC	
Calyptranthes concinna DC	
Ochnaceae	
Ouratea sp1	
Orchidaceae	
Prosthechea vespa (Sw.) W. E. Higgins	
Poaceae	
Trachypogon spicatus (L. F.) Kuntze	
Rubiaceae	
Posoqueria latifolia (Rudge) Schult.	
Barreria sp1	
Theaceae	
Gordonia fruticosa (Schrad.) H. Keng	
Velloziaceae	
Barbacenia flava Mart. ex Schult. F.	
Vellozia albiflora Pohl	

the opposite: pollinators were more specialized in plants than the plants on pollinators.

This result can be explained for differences in environmental complexity between the two physiognomies. The Riparian Forest and the Rocky Grassland present distinct vegetation patterns, sharing few plant species in common and exhibiting different microclimatic conditions [37]. The Riparian Forest is always more humid and shady, while the Rocky Grassland is more sunny and presents wider variation in the thermal amplitude and in the yearly and daily humidity, as a general reference. The greater complexity of the interaction network concerning flower-visiting social wasps found in the Riparian Forest can also be explained by the heterogeneity of the vegetation at this site and wider diversity of niches. The Riparian Forest in Ibitipoca State Park is characterized by a sequence of physiognomies with shrubby arboreal to predominantly arboreal formations near water sources [37], which is ideal for wasps to build its nests.

Contrastingly, the Rocky Grassland presents a structure with a wide diversity of herbs, grasses, and shrubs distributed over quartzite outcrops [37]. Santos [36], studying communities of social wasps associated with a mangrove swamp, the Atlantic Forest, and the Restinga Forest (coastal plain vegetation), reported that the diversity of wasps found in each of these tropical ecosystems was significantly correlated with the diversity of plants because they provide more substrates for nesting [52, 53], more amount of food recourses (i.e., nectar [25, 28, 54]), more materials for nest construction (i.e., plant fibers [55, 56]), and more areas for prey foraging [57]. Therefore, the vegetation complexity is of decisive importance in the composition and structure of the social wasp communities, directly influencing their niches and their associations [36]. In this manner, the differences found in the two networks evaluated here may reflect the landscape characteristics, which are responsible not only for the increase in the richness and abundance of the interactions in the environments, but also for the establishment of a more generalist or specialist pattern in its interaction network with plants.

On the other hand, although the environmental complexity is related to the abundance of individuals and species, the behavioral characteristics of the species also have to be regarded as a relevant factor in the network composition and structure. Species such as *Protonectarina sylveirae* (Saussure, 1854), *Polybia sericea* (Olivier, 1791), *Polybia paulista* (Ihering, 1896), *Apoica pallens* (Fabricius, 1804), *Brachygastra lecheguana* (Latreille, 1824), *Polistes canadensis* (Linnaeus, 1758), and *Polistes ignobilis* (Haliday, 1836) present a wider range of ecological tolerance than other species and are generally dominant in open ecosystems, with standing severe environmental conditions such as in the Rocky Grassland [36].

In this context, the complexity of the two phytophysiognomies, as well as the behavioral characteristics of the species involved in the interactions between flower-visiting social wasps and plants, is an important factor to establish the network patterns found in the present study. Hence, an environment of greater niche complexity and plant species diversity provides better conditions for the maintenance of more complex networks, where interactions are concentrated on generalist species, thus forming a network with a nested structure and asymmetric specialization. A recent study showed that species diversity influences the network pattern [58]. In this study, the author showed that sites with greater richness and diversity of species tend to overlap connections in network and consequently increase the generalization of network interactions. Conversely, environments with reduced complexity may shape interactions by making them more specialized [29], as evidenced in the Rocky Grassland network. However, this result was not previously tested for interactions between social wasps and plants.

The variation in the values of niche amplitude found in this study for wasps may be related to factors that influence the foraging activity [57]. Social wasps have in floral nectar its main food source [59, 60], what may be associated to the facility to access this resource compared with the energy spent to search, subdue, and capture a prey [61]. This fact is more evident in species bearing small colonies like *Mischocyttarus*, the most abundant and with higher niche amplitude group in this study. On the other hand, differences in the biomass of some species may also influence foraging activities of social wasps [57]. Populations with higher biomass use a greater amount of food resources [28]. This could explain the wider foraging spectrum of genus *Polybia* and also greater abundance of visiting individual flowers. These wasps build large nests that have many individuals. According to Spradbery [62], size can be a decisive factor for resource consumption, affecting the foraging amplitude of these species, and the colony productivity may influence the foraging activity.

An additional factor determining the foraging pattern of social wasps is plant-flowering phenology, what directly influences resources abundance and distribution [2], see also [63]. A higher diversity of plant species may increase over time the variety, quality, and availability of resources for flower visitors, like wasps [28, 64, 65]. The niche amplitude as well as the specialization degree should be directly related with environment characteristics, including its seasonal variations (see [66]).

Although more than half of the plants were visited by only one to three individuals and wasp species, leading to a low importance index for most plant species in both areas, this result corroborates the observations by Heithaus [1] and Santos et al. [28]. Nevertheless, some plant species may be visited very frequently, as observed to Asteraceae, which was involved in most interactions. This result may be related to the wide distribution of this family species in the Rocky Grassland and in the Riparian Forest of Ibitipoca State Park. Furthermore, this frequent visitation to Asteraceae is also probably due to the anatomy of their flowers, which present a brush-like inflorescence with a great number of small compact flowers, making them more conspicuous to insects [67]. Asteraceae species usually have bright colored yellow or white flowers, effectively attracting insects [68], and they also present abundant amounts of nectar, which is located at a depth of just a few millimeters, ideal for the size of wasp mouthparts [68, 69].

The flowers of the *Baccharis* sp1 were the most visited considering the abundance of individuals and richness of species in the two phytophysiognomies, showing them to be of great importance to the interaction network between social wasps and plants of Ibitipoca. In southern Brazil, Hermes and Köhler [10] observed that 28% of the individual wasps collected were found on flowers of *Baccharis*, the second most visited genus by this species of social wasp. These researchers also reported that *M. drewseni* females were captured mainly on *Baccharis tridentata* and *Baccharis myriocephala*. In a general context, although social wasps are rarely regarded as pollinators, with varying effectiveness according to the species associated [70], they act as regular visitors to flowers in various environments. Thus, the conservation of these wasp species may be relevant to the maintenance of diversity in natural communities.

Currently, research into networks in interaction studies is beginning to attach importance to the factors that shape the properties of interaction networks. However, most studies involving networks of interactions between organisms still relate the network pattern only to the type of interaction established between the groups [15, 16, 71, 72]. Our results, despite having a small sampling of the wasp-plant interaction network, suggest that differences in structural complexity of the environment directly influence the structure of the interaction network between flower-visiting social wasps and plants. Hence, knowing the biotic and abiotic factors of the sites in which such interactions take place is of fundamental importance to define and understand the structure of the networks established in these areas. Therefore, research concerning network patterns of different types of interactions established between organisms contributes to a more complete understanding of the ecology of communities in distinct environments.

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Research Article

Diversity and Nesting Substrates of Stingless Bees (Hymenoptera, Meliponina) in a Forest Remnant

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Stingless bees are abundant and diverse key actors in several plant-pollinator networks in the neotropics, but little is known about their natural history and ecology. This study aims to contribute to knowledge about the diversity and dispersion of stingless bees and discusses the importance of nesting substrates. It was carried out in the Araguari river valley in Minas Gerais, Brazil, where a nest site survey was conducted in an area of 100 ha during 11 alternate months from 2006 to 2008, for a total of 1,200 observation hours. Sixty-nine nests were found, belonging to 12 genera and 20 different species. Nests of *Melipona rufiventris* were by far the most abundant. Stingless bees nested more frequently in hollows of live trees (64%), and 11 different substrates were identified. Seventeen plant species were used as nesting substrates and *Anadenanthera macrocarpa* (Fabaceae) was the main host, encompassing 23% of the surveyed nests. The area studied is important for the maintenance of stingless bees because it provides nesting sites for them. Without nesting sites the reproductive division of colonies is compromised, affecting the nests' survival.

1. Introduction

Insects comprise the most diverse animal group on the planet, with about 925,000 known species worldwide [1]. They stand out by presenting several adaptations, interacting with various groups of animals and plants and having a large biomass and a wide niche breadth. An important mutualistic interactive mechanism between plants and insects is pollination [2, 3], and eusocial bees are responsible for 30 to 50% of these interactions [4].

The number of bee species in the world is about 30,000 [5, 6], and Brazil accounts for an estimated 10% of this total [7]. The high diversity of bees in Brazil is mainly owed to the tropical climate, which permits perennial nests, and the abundant supply of food. Stingless bees, native to Brazil, are represented by more than 200 species [7] and have a high commercial value because they are pollinators of several native and exotic crops [8, 9]. Knowledge of the basic ecology of bees is essential for underpinning the development and implementation of conservation strategies [10] and the development of management techniques favoring commercial farmers. In this context, ethological studies on aspects such as foraging ecology, activities such as collecting food resources, material for nests, the demand for sexual partners, and the choice of sites for nests or shelter are extremely relevant.

The choice of nesting site is a stage in the swarming process. The division of reproductive nests in stingless bee colonies starts with a visit by the workers to potential sites for a nest in the vicinity of the parent colony [11]. Most species nest in hollow trees, but many use termite mounds (active or abandoned), anthills, gullies, and crevices in walls, among other places [12]. Nests can be found in different forest environments, such as the Brazilian savanna (Cerrado biome).

The Cerrado is a very old domain. Since the Cretaceous (between 145 and 65 million years ago), there was a pre-cerrado formation. Soon after this period, the uplift of the central plateau and a gradual climate change, to a wetter period, promoted the diversification of flora and fauna [13]. The Cerrado biome comprises a mosaic of types of vegetation, ranging from grassland to dense woodland "cerradão". Throughout this gradient of vegetation there is a gradient of biomass, conditioned by natural factors that determine the carrying capacity of the environment and/or by human pressures, especially grazing, frequent fires, and exploitation of wood [14]. The Cerrado covers approximately 22% of Brazilian territory and has suffered significant human impacts during the past three decades [15], whereas only 1.5% of this area is protected in conservation areas [16]. One of the regions which has suffered the worst human impact is the westernmost part of the state of Minas Gerais, known as Triângulo Mineiro and located from 18 to 20°S; 47 to 51°W, where up to 90% of the original cover has been wiped out. In this region, native vegetation has been replaced by pastures and cultivated areas [17], or drastically reduced and fragmented. Anthropogenic disturbances influence most organisms, including native bees and other pollinators [18].

Ecological perturbations resulted in a marked turnover of nesting resources, leading in large shifts in the relative proportions of nesting guilds [19]. The abundance of stingless bees' nests is usually related to the availability of appropriate nesting sites, which may act as a limiting resource for these species [20–22]. Other factors that negatively influence stingless bee populations are invasion by exotic species and expansion of agriculture [23], which may even lead undescribed species to extinction [24]. Bees are efficient pollinators contributing to fruit and seed production in different ecosystems [8]. Actually, they may account for 40 to 90% of native plant pollination, depending on the ecosystem [25, 26].

Compared to existing information for areas of temperate forest, knowledge of intra and interspecific ecological relationships that occur in the Cerrado is scarce, as this environment little explored regarding interactive biodiversity [27]. Several studies describe the fauna of stingless bees in the Cerrado through the evaluation and sampling of indivduals visiting floral resources [28–32]. Only a few studies, however, evaluate nest distribution and nesting habits of stingless bees in urban [33–35] and natural areas [35–38]. Although there are several threatened native vegetation remnants in the Triângulo Mineiro region, mostly owing to deforestation and agriculture, there is a lack of information on bee diversity, species composition, and the importance of the plants in their food collection.

To contribute to knowledge about stingless' bees diversity and dispersion, this study describes the nesting sites occupied by various species and discusses the importance of these substrates in the dispersal and maintenance of the species.

2. Material and Methods

2.1. Study Area. This study was carried out in the Araguari river valley (18°37′, 48°19′), in the municipality of Araguari, Minas Gerais, south-eastern Brazil (Figure 1). The climate is tropical wet (Aw in Köppen's system [39]), and there is a rainy season from October to March and a pronounced dry season from April to September [40]. Annual rainfall ranges from 1,300 to 1,700 mm, and mean monthly temperature shows little variation throughout the year [41].

A nest site survey was conducted in an area of 100 ha that was previously covered mostly with deciduous forest, but there were also a few stretches covered with gallery and semideciduous forests. This area was contiguous to a grazing area, where there were old buildings and cement and wooden poles. The study site is located between 532 and 730 meters above sea level.

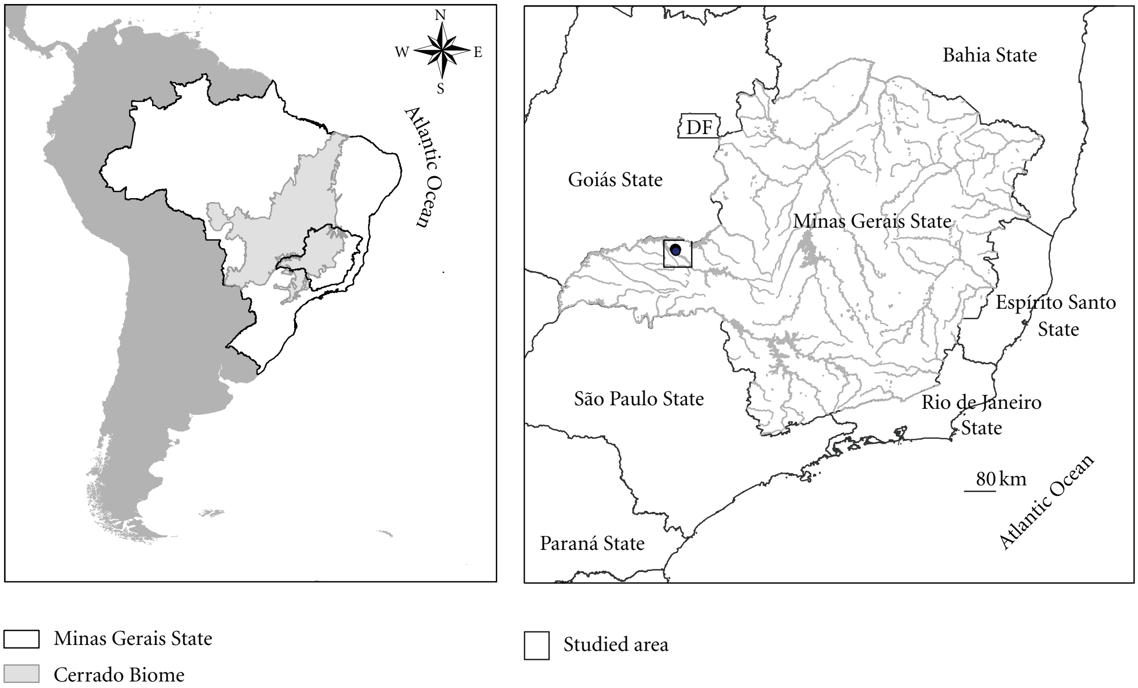
2.2. Nest Site Surveys of Stingless Bees. The study was carried out between 2006 and 2008, in 11 alternate months and 1200 observation hours, following random lines established for fauna rescue actions before cleaning and flooding for a dam for hydroelectric power generation. Nest site location was conducted through direct visual search, and each nest position was registered as a geographical coordinate with Global Positioning Systems (GPS).

Nest site location was conducted through direct visual search of tree trunks and termitaria, which were inspected from the base to ten meters' height. The substrate type used for nesting was recorded, and, whenever possible, the nests were transferred to other areas, in order to protect bees against flooding. A few nests which could not be directly transferred to other areas without suffering damage were transferred to wooden boxes and kept in the experimental garden of the Instituto de Biologia (INBIO) at Universidade Federal de Uberlândia (UFU). Voucher specimens of stingless bees and exsiccates of host plants were deposited in the Museu de Biodiversidade do Cerrado (UFU) and Herbarium Uberlandense (UFU, Uberlândia, Minas Gerais, Brazil), respectively.

The entrance of each nest was described and photographed to allow stingless bee species identification. Moreover, individuals belonging to worker castes were also collected and deposited in the following reference collections: Laboratório de Ecologia e Comportamento de Abelhas (LECA) of UFU, Entomological Collection of the Taxonomic Collections of the Universidade Federal de Minas Gerais (UFMG) and Hymenopteran Collection of Faculdade de Filosofia, Ciências e Letras of the Universidade de São Paulo, Campus of Ribeirão Preto (RPSP).

2.3. Data Analysis. Stingless bee species diversity and evenness were calculated with the Shannon-Weaver index (H') and Pielou index (J'), respectively [42]. In order to detect the type of spatial pattern, the Johnson and Zimmer index of dispersion (I) [43] based on the distance between the sampled points plotted on the map was used.

The precise location of each nest was mapped with ArcView GIS to determine patterns of nest dispersion. We assigned each nest to a substrate type and counted the number of nests belonging to each substrate local. The following substrate types were used: live tree trunk, dead tree trunk, rock substrate, soil, epigeous termitaria, arboreous termitaria, underground termitaria, cement pole, wooden pole, vine, and wall.



(a) (b)

Figure 1: (a) Location of the state of Minas Gerais in Brazil and original extension of Cerrado; (b) Study site location in Minas Gerais.

In order to evaluate patterns of nesting substrate used by the bees, we performed a Principal component analysis (PCA), which ordinates substrate type categories per bee species, using PC-ORD 5.10 version [44]. According to Gauch [45], the first principal component accounts for most variability in the data, and each succeeding component accounts for as much of the remaining variability as possible. In this way, each axis produces an eigenvalue, which corresponds to the relative contribution of that component in explaining the total variance in dataset.

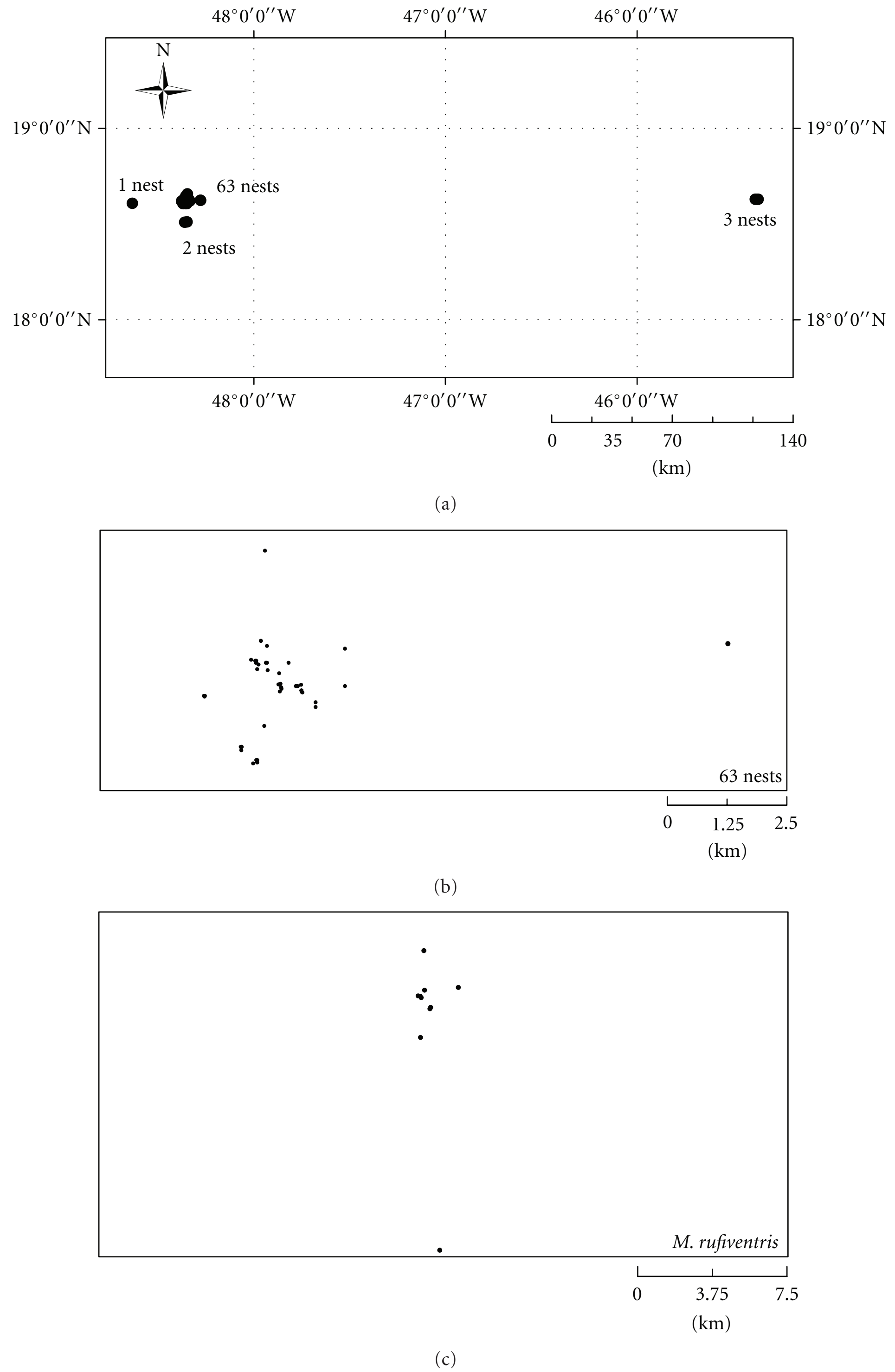
3. Results

We found 69 nests of Meliponina bees at the study site, belonging to 12 genera and 20 different species. The bees occupied 11 distinct substrate types (Tables 1 and 2). The genus *Trigona* presented the highest number of species (six) in the study site, followed by the genera *Tetragona*, *Partamona,* and *Scaptotrigona*, represented by two species each. Regarding nests, *Melipona rufiventris* Lepeletier, 1836 was the species with highest abundance ($N = 10$ nests), followed by *Tetragonisca angustula* Latreille, 1811 ($N = 7$ nests) and *Scaptotrigona bipunctata* Lepeletier, 1836, *Tetragona clavipes* Fabricius, 1804, *Trigona hyalinata* Lepeletier, 1836, and *Partamona ailyae* Camargo, 1980 ($N = 6$ nests) (Table 1).

The diversity of nests of stingless bee species in the study site was 2.68 (H'), and evenness (J') was 0.89, which characterizes a high species' evenness. The 69 nests mapped presented a strongly clumped distribution, since the Johnson and Zimmer index of dispersion (I) was 10.2. This index was calculated for *M. rufiventris*, the most abundant species, and the nests showed a clumped distribution ($I = 3.32$) (Figure 2).

Tetragonisca angustula used a higher number of substrate types (dead tree trunk, live tree trunk, wooden pole, and wall), representing 36.4% of the total, followed by *Frieseomelitta varia* Lepeletier, 1836 which used 27.3% of different substrate types (wall, cement pole, and wooden pole) (Table 1). Some species, however, nested in specific substrates, such as *M. rufiventris*, which used exclusively tree hollows (mostly from live trunks, which represented 90%). *Cephalotrigona capitata* Smith, 1874, *Scaptotrigona bipunctata* Lepeletier, 1836, and *Tetragona clavipes* Fabricius, 1804 nested exclusively in live trunks. On the other hand, *Partamona combinata* Pedro and Camargo, 2003, *Trigona recursa* Smith, 1863, and *Trigona fulviventris* Guérin, 1837 nested only in sites associated with termites (Table 1). Most nests were located in hollows of live trunks (64%), which were occupied by 14 different stingless bee species (Table 2). Seventeen plant species were used as substrate for nesting, and *Anadenanthera macrocarpa* (Benth.) Brenan (Fabaceae) was the commonest one (Table 3).

The Principal component analysis (Figure 3) pooled substrates in at least two major groups, according to distance from ground level, showing similarities between nesting habitats of some species of bees. The first group was composed of substrates located at ground level (rock, dead trunk, and underground termitaria), which presented similar values for both ordination axes. Substrates located above ground (cement pole and wooden pole) formed a second group, situated at the center of the ordination. The ecological



(a)

(b)

(c)

Figure 2: (a) Spatial distribution of the 69 nests of stingless bees in the study area mapped. (b) Spatial distribution of the 63 nests presented in Figure 2(a), on a large scale. (c) Spatial distribution of the 10 nests of *M. rufiventris* mapped in the study area.

preference for live trunk substrate was found in the analysis, through the placement of this category in the central portion of the ordination. On the other hand, substrates that were seldom used (e.g., arboreus termitaria, murundum termit and vines) presented lower values from the second axis, and failed to form groups with most substrates present in the ordination. The first axis explained 61.8% of the variance in the dataset, while the second axis explained only 7.21%.

Table 1: Number of nests and substrate types used by each stingless bee species in a forest fragment located in the municipality of Araguari, Minas Gerais, Brazil.

Bee species	Number of nests	Nesting substrate category*
(1) *Cephalotrigona capitata* (Smith, 1874)	03	f, f, f
(2) *Frieseomelitta varia* (Lepeletier,1836)	03	e, g, h
(3) *Lestrimelitta limao* (Smith,1863)	01	f
(4) *Melipona rufiventris* (Lepeletier, 1836)	10	b, f, f, f, f, f, f, f, f, f
(5) *Oxytrigona tataira* (Smith, 1863)	01	f
(6) *Partamona ailyae* (Camargo, 1980)	06	f, f, f, f, f, k
(7) *Partamona combinata* (Pedro and Camargo, 2003)	03	j, j, k
(8) *Plebeia droryana* (Friese, 1900)	01	f
(9) *Scaptotrigona bipunctata* (Lepeletier, 1836)	06	f, f, f, f, f, f
(10) *Scaptotrigona depilis* (Moure, 1942)	04	b, f, f, f
(11) *Scaura longula* (Lepeletier, 1836)	01	f
(12) *Tetragona clavipes* (Fabricius, 1804)	06	f, f, f, f, f, f
(13) *Tetragona quadrangula* (Lepeletier, 1836)	02	f, f
(14) *Tetragonisca angustula* (Latreille, 1811)	07	b, e, f, f, f, f, h
(15) *Trigona fulviventris* (Guérin, 1837)	02	c, c
(16) *Trigona hyalinata* (Lepeletier, 1836)	06	a, f, f, f, f, f
(17) *Trigona hypogea* (Silvestri, 1902)	02	d, f
(18) *Trigona recursa* (Smith, 1863)	02	k, k
(19) *Trigona spinipes* (Fabricius, 1793)	02	i, i
(20) *Trigona truculenta* (Almeida, 1985)	01	a

*a: rock substrate, b: dead trunk, c: underground termitaria, d: soil, e: wall, f: live trunk, g: cement pole, h: wooden pole, i: vine, j: arboreous termitaria, k: epigeous termitaria.

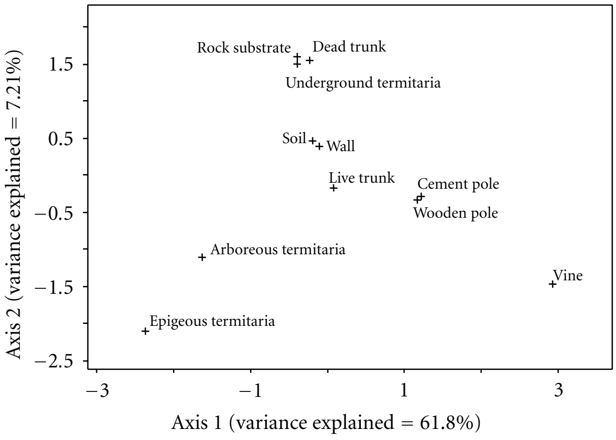


Figure 3: Principal component analysis (PCA) ordinating substrate types used for nesting per twenty stingless bee species that occurred in a forest fragment located in the municipality of Araguari, Minas Gerais, Brazil.

Table 2: Number of stingless bee nests for each substrate type, and number of species nesting for each substrate in a forest fragment located in the municipality of Araguari, Minas Gerais, Brazil.

Substrate type	Number of nests	Number of species using the substrate
(1) Cement pole	1	1
(2) Dead trunk	6	3
(3) Epigeous termitaria	4	3
(4) Live trunk	44	14
(5) Rock substrate	2	2
(6) Soil	1	1
(7) Arboreous termitaria	2	1
(8) Underground termitaria	3	1
(9) Vine	2	1
(10) Wall	2	2
(11) Wooden pole	2	2

4. Discussion

Different substrates were used by stingless bees as shelters in which to nidify inside the dry forests that include different physiognomies contiguous to a grazing area. This characteristic of the dry forests may account for the abundance of stingless bee samples whereby the nesting success includes the abundance, size, and species of trees [46, 47]. Suitable nesting sites are considered limiting resources for stingless bees [21, 25], whereas the availability of materials used in nest construction, food supply, and natural enemies [48] is also important for the survival and reproduction of species. These factors also appear to be relevant for other social invertebrates such as ants [49] and pseudoscorpions [50].

Owing to the peculiar swarming activity in Meliponina, which limits maximum flight distance, the distance between appropriate nesting sites, and the connectivity between

Table 3: Plant species used as nesting substrate by stingless bee species, and number of nests per bee species in each different plant in a forest fragment located in the municipality of Araguari, Minas Gerais, Brazil.

Plant species	Stingless bee species	Nests number/stingless bee species/plant	Total nests/plant
(1) *Anadenanthera macrocarpa* (Benth.) Brenan (Fabaceae)	*Cephalotrigona capitata*	1	16
	Lestrimelitta limao	1	
	Melipona rufiventris	1	
	Oxytrigona tataira	1	
	Partamona ailyae	3	
	Scaptotrigona bipunctata	3	
	Scaptotrigona depilis	1	
	Tetragona clavipes	3	
	Tetragonisca angustula	1	
	Trigona hyalinata	1	
(2) *Caryocar brasiliense* Camb. (Caryocaraceae)	*Melipona rufiventris*	1	2
	Tetragona quadrangula	1	
(3) *Chorisia speciosa* A. St.-Hil. (Malvaceae)	*Trigona hyalinata*	2	2
(4) *Copaifera langsdorffii* Desf. (Fabaceae)	*Scaptotrigona bipunctata*	1	2
	Tetragonisca angustula	1	
(5) *Dipteryx alata* Vogel (Fabaceae)	*Tetragonisca angustula*	1	1
(6) *Ficus* sp. (Moraceae)	*Melipona rufiventris*	1	2
	Tetragonisca angustula	1	
(7) *Guapira* sp. (Nyctaginaceae)	*Cephalotrigona capitata*	1	4
	Melipona rufiventris	1	
	Plebeia droryana	1	
	Scaptotrigona bipunctata	1	
(8) *Hymenaea courbaril* L. (Fabaceae)	*Tetragona clavipes*	1	3
	Trigona hyalinata	2	
(9) *Myracrodruon urundeuva* Fr. All. (Anacardiaceae)	*Partamona ailyae*	1	2
	Trigona hypogea	1	
(10) *Pouteria ramiflora* (Mart.) Radlk. (Sapotaceae)	*Melipona rufiventris*	1	1
(11) *Qualea parviflora* Mart. (Vochysiaceae)	*Cephalotrigona capitata*	1	1
(12) *Sclerolobium aureum* (Tul.) Benth. (Fabaceae)	*Melipona rufiventris*	1	1
(13) *Senna rugosa* (G.Don) H.S. Irwin and Barneby (Fabaceae)	*Scaura longula*	1	1
(14) *Stryphnodendron adstringens* (Mart.) Cov. (Fabaceae)	*Melipona rufiventris*	1	1
(15) *Tabebuia aurea* (Manso) Benth. and Hook.f. ex S. Moore (Bignoniaceae)	*Melipona rufiventris*	1	3
	Partamona ailyae	1	
	Tetragona clavipes	1	
(16) *Tapirira guianensis* Aubl. (Anacardiaceae)	*Tetragona clavipes*	1	1
(17) *Terminalia argentea* Mart. et Zucc. (Combretaceae)	*Tetragona quadrangula*	1	1

fragmented habitats directly influence the dispersion ability of bees and species-genetic variability. Disparate forest fragments may attract only limited colonization by stingless bees because, during the swarming process, the colony that is formed depends on food and materials for nest construction provided by the maternal nest for some days [5, 51]. Consequently, to allow successful dispersal, nesting sites should be within worker bees' flight range, and well-preserved fragmented areas should be connected. The present study confirmed the importance of maintaining the study area that functions as a provider of nest sites for stingless bees. This is a fundamental condition for the survival of these species, because otherwise the reproductive division of the colony is compromised, affecting nest survival and consequently the dispersion of the species. Thus, the maintenance of natural areas close to each other is essential.

Although 17 plant species were used as nesting substrate, *Anadenanthera macrocarpa* was the main substrate used by nesting bees. The suitability of *A. macrocarpa* as a nesting substrate may be related to its large trunk diameter, which presents larger natural cavities in contrast to most Cerrado plant species that present thin and contorted trunks. The preference for some plant species is reported by previous authors, in that more than 61% of nests were encountered in only two species of tree [36, 38]. The diversity of bee genera found in this study represents 60% of all genera occurring in Minas Gerais state, and as regards species this value is about 41% compared with data presented in a systematic study of stingless bees by Silveira et al. [7].

This study was carried out in a forest remnant that includes at least three different physiognomies presented in such a way that habitat heterogeneity contiguous to a grazing area may account for the abundance of stingless bee samples. This characteristic could explain the bee species' richness and the nest dispersion, which provides a wide variety of nesting sites and food resources, thus facilitating species' coexistence through resource partitioning. Stingless bees constructed their nests mostly in hollows of live tree trunks; hence, deforestation greatly influences their community structure and population dynamics [52] owing to a decrease in the availability of nesting sites and food supply.

Studies which try to explain the diversity of stingless bees through a search for nests are scarce because of difficulties imposed by the search method, which can actually increase depending on the vegetation and topography of the area in question. Environments consisting of low and open vegetation the nests can be found more easily. However, in high vegetation with closed canopy the application of this method becomes more difficult.

The aggregated pattern of distribution of nests should be correlated with the composition of existing vegetation in the study area. The nests were mostly found in places where there are trees with thicker trunks, in semideciduous forest in the savanna and in areas adjacent to pastures, where many trees have been preserved. The same is true of *M. rufiventris*, whose nests were found mainly near streams in areas of gallery forest (Siqueira, pers. comm.). In studies of *Trigona collina*, species in a mixed area of deciduous forest in Thailand had a strongly clumped nest distribution [37]. Serra et al. [38], studying three different areas of disturbed savanna vegetation, found for two of them a random pattern that was understood as the absence of competition for food resources. The pattern of spatial distribution of nests is possibly related to extrinsic factors such as distribution and density of suitable substrates for nest construction, the ecological aspects of occupancy of hollows, predation [38] and related features of the swarming process [37].

According to Laroca [53], bee communities usually follow a lognormal distribution, in which a few species are rare or highly dominant, and most species present moderate abundance. Our data on nest abundance of stingless bees fit with this species-abundance relationship.

Some studies evaluated stingless bee nest diversity in Brazilian Cerrado areas. In the Araguari river valley, nest diversity was higher than that found by Serra et al. [38] and Mateus et al. [35]. Serra et al. [38] studied a Cerrado area of 114 ha in the state of Maranhão (north of Brazil) and found 40 nests belonging to ten Meliponina species. In a natural Cerrado area in Nova Xavantina (Mato Grosso, central Brazil), Mateus et al. [35] found 34 nests belonging to seven genera and 12 species. Maia et al. [54] found seven different species nesting in trunks in a gallery forest area in a Cerrado area in the state of Maranhão (north of Brazil). In the latter study, *M. rufiventris* was more abundant than other species, which is in accordance with our results.

Carvalho and Bego [29] sampled bees visiting floral resources in a Cerrado area near our study site and found nine genera and 11 Meliponina species. Considering the methodological differences and the difficulty of finding bee nests in the wild, our results suggest that bee species' richness is almost twice as high in our study site as that of Carvalho and Bego [29], which reinforces the importance of protecting our study site.

M. rufiventris is considered a specialist in flowers occurring in well-preserved areas [55], and it presents a strong connection with water courses, as nests are usually found close to or along river margins [56]. This species, along with *P. combinata*, was in the IUCN Red Lists for the state of Minas Gerais [57]. In addition to habitat specialist species, the Araguari river valley stingless bee community retains several rare species, including *Melipona quadrifasciata*, *Melipona quinquefasciata*, *Partamona helleri*, *Plebeia remota*, *Nannotrigona testaceicornis* and *Schwarziana quadripunctata* (Siqueira, pers. comm.). These species were added to the community through sporadic observations of bees visiting flowers. Hence, we suggest that (1) the study site is important in terms of maintaining stingless bee species' diversity, (2) future studies using methods ranging from nest surveys to direct collection of bees visiting flowers could increase the observed Meliponina species' richness in the study site, and (3) management plans and conservation actions should be employed in order to protect the diversity of stingless bees in Cerrado biome.

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Research Article

Use of Plant Resources by *Merosargus* (Diptera, Stratiomyidae, Sarginae) Larvae

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The genus *Merosargus* (Loew) has 142 described species. This great diversity in the genus could be explained by larvae resource-use specialization. However, information on larval habitats is still very scarce. In *Merosargus* species, adult males defend oviposition sites, and this territorial behavior may lead to interspecific competition and make even more important the specialization and niche partitioning to prevent competitive exclusion. This study identified substrate types used as a resource by *Merosargus* larvae and investigated the degree of specialization and overlap in resource use by different species at an Atlantic forest remnant in Minas Gerais, Brazil. Every potential resource, especially those with adults in the vicinity, was collected opportunistically from October 2001 to October 2004. In total, 292 individuals from 12 *Merosargus* species collected from 21 resource types and 15 plant species were reared in the laboratory. Plant species included herbs, vines, palms, and trees. Six *Merosargus* species were reared from only one resource type, and each resource type was used, on average, by less than two *Merosargus* species. Thus, *Merosargus* species exhibited a high degree of specialization and small overlap in larval resource selection, which could explain the high local and global diversity of the genus.

1. Introduction

The family Stratiomyidae (soldier flies) occurs in all warm temperate and tropical regions, especially in the wet tropics [1], and includes species with great morphological and habitat use diversity [2].

Little is known about Stratiomyidae larval biology in the Neotropical region. The larval stages of soldier flies have been extensively studied over the last decade in Brazil, but the studies focused mainly on larval and pupal morphology [3–12].

The genus *Merosargus* (Loew) is one of the largest in number of species in the family Stratiomyidae. Woodley [2] listed 142 species in the genus, and only two are not Neotropical. The previous revision of the genus is over 40 years old and listed only 109 species [13], thus the genus needs to be reviewed.

The biology of the genus is still poorly known. Woodley [2] reported the occurrence of *Merosargus* adults around various types of fruits and other rotting plant material on the forest floor, where males defend small territories, females oviposit, and mating occurs. He hypothesized that some species specialize on certain plant resources. Therefore, resource-use specialization by larvae could be an explanation for the great diversity in the genus. However, information on larval habitats is still very scarce and not species-specific [14–18].

In the Neotropical region, a wide variety of resources is available for phytophagous insects. Adult insects must recognize and select the best available oviposition substrates, and, for many species, resource quality has a major effect on their distribution and abundance [19]. The choice of oviposition sites is crucial for larval development and adult survivorship and vigor. In fact, several factors such as nutritional quality, predation risk, and competition may act directly or indirectly in oviposition site selection [20].

Specialization in resource use is a way to maintain the coexistence of a large number of closely related insect species in tropical forests. However, recent studies have shown that different insect herbivore guilds exhibit different degrees of specialization and that some guilds such as chewers are predominantly generalists [21]. In addition, scavengers such as litter arthropods are often assumed to be generalists because they harvest nutrients from dead plant material and litter-decomposing microbes rather than directly interacting with living plants [22].

Conversely, specialization could be more important in cases where territorial defense occurs. Oviposition site defense is typical of species that use ephemeral resources and where females mate multiple times [23]. In these cases, last male sperm precedence is a common pattern [23, 24], and this behavior has been demonstrated in one *Merosargus* species [25]. Therefore, the defense of oviposition sites in *Merosargus* is very important to ensure mating just before oviposition. In fact, this behavior has already been observed in ten of the 18 *Merosargus* species found in our study site. Resident males avidly guard their territories repelling all intruders, including males of their own species or males and females of other species (Fontenelle et al. unpublished data). Therefore, strong interference competition could occur among *Merosargus* species using the same oviposition sites, which could lead to the exclusion of less combative species in the absence of resource use differentiation.

The understanding of oviposition site selection in *Merosargus* could not only explain the maintenance of high species diversity in the genus but also its origin, because assortative mating may occur in cases where males and females are attracted to a particular resource, which may have a prominent role in ecologically-driven sympatric speciation [26].

Thus, this study aimed to (i) identify the plant substrates used as a resource by *Merosargus* larvae, (ii) determine the period of the year when these substrates are used, (iii) investigate whether there are differences in the degree of specialization in resource use by different species, (iv) investigate whether there is an overlap in resource use by different species, and (v) determine which species use these resources similarly. Assuming that territorial defense leads to strong interspecific interference competition, we expect to find a high degree of specialization and low similarity in oviposition site selection among sympatric *Merosargus* species.

2. Methods

2.1. Merosargus Sampling and Rearing. Parque Estadual do Rio Doce (hereafter PERD), located between 19°48′18′′–19°29′24′′ S, 42°38′30′′–42°28′18′′ W, is an important Atlantic forest remnant in southeastern Brazil [27]. The PERD has an area of approximately 36,000 ha with a complex pattern of vegetation types [28]. The climate is humid, tropical, and mesothermal, with the rainy season from October to March and dry season from April to September [29].

At least six Sarginae genera (*Merosargus*, *Ptecticus*, *Sargus*, *Microchrysa* Loew, *Acrochaeta* Wiedemann, and *Himantigera* James) and 18 *Merosargus* species have already been recorded in the PERD (Fontenelle et al. unpublished data).

This study was conducted opportunistically from October 2001 to October 2004. Sampling was done simultaneously to field campaigns from other studies that used malaise traps and that investigated the territorial behavior of *Merosargus* species associated to *Heliconia* conducted during this period. Therefore, the sampling effort was not the same for all periods of the year or all visited areas.

Samples were collected at six sites composed of semideciduous forests at different regeneration levels. We also sampled substrates in a forest fragment near the PERD with the same vegetation type, but an impoverished vertebrate fauna.

Every substrate that could theoretically be used by Sarginae larvae was collected. The sighting of adults on substrates or around their vicinity was considered the strongest evidence of larval occurrence. After first spotting adults on a particular substrate type that substrate was collected at subsequent field trips, even in the absence of adults, to better determine the larval occurrence period. A sample was considered a particular substrate type (plant structure) collected in the same place at the same date, often corresponding to a large amount of substrates.

The substrates were taken to the laboratory and kept in sealed chambers at room temperature until adults emerged. This paper only reports on collected substrates containing Sarginae larvae that resulted in the emergence of adults. Adult Sarginae were identified to genus level using the Woodley [30] identification key. *Merosargus* adults were identified to species level using James and McFadden's [13] key. Several species reared in our study could not be identified with certainty because taxonomic revision is needed for the genus *Merosargus*. Therefore, we chose to use the species names that are still uncertain only as a reference for possible species or the closest species; these species were, respectively, indicated in the result tables with "cf." or "aff." before the specific epithet. Voucher specimens were deposited in the Laboratório de Pesquisas Ambientais CODAAMB/IFMG-OP.

2.2. Data Analysis. Plant species and resource type specificity were determined using a host specificity index (H) [31]. This index is an estimate of the proportion of hosts used by a particular *Merosargus* species among all hosts available minus one

$$H = \frac{(S_F - 1)}{(S_T - 1)}, \tag{1}$$

where S_F is the number of plant species (or resource types) used by a *Merosargus* species, and S_T is the total number of plant species available. However, we modified the index replacing S_T by S_U, the total number of plants used by any *Merosargus* species. This was done because it was impossible to determine the actual availability of plant species as food

Table 1: Plant species and structure used as a resource by *Merosargus* larvae, month of occurrence, and number of *Merosargus* species found in samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004.

Plant species	(Structure)	Month of occurrence												Number of *Merosargus* species
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
Panicum maximum	(Pseudostem)	×												1
Heliconia spathocircinata	(Inflorescence)	×												2
	(Pseudostem)		×					×						2
H. episcopalis	(Inflorescence)	×		×	×									2
	(Pseudostem)				×			×	×		×	×		5
	(Bored pseudostem)								×					2
H. aemygdiana	(Pseudostem)		×											1
Thoracocarpus bissectus	(Stem)		×					×				×		4
Amaranthaceae sp.2	(Leaf)			×										1
Heliconia x matenensis	(Inflorescence)			×										1
	(Pseudostem)			×								×		1
Urera sp.	(Stem)				×									2
Cucurbitaceae sp.1	(Leaf)				×							×		1
Amaranthaceae sp.1	(Leaf)					×								2
Guarea sp.	(Fruit)					×			×		×			1
Astrocaryum aculeatissimum	(Leaf)						×		×				×	3
	(Stem)								×					2
Euterpe edulis	(Stem)								×					1
Musa sp.	(Inflorescence)								×					2
	(Pseudostem)								×					1
Lecythis lurida	(Flower)												×	1
Number of resources/months		3	4	4	4	2	1	3	8	0	3	4	2	$S_{\text{avg}} = 1.81$
**Joannesia princeps*	(Fruit)		×		×									0
**Spondias* sp.	(Fruit)				×									0
*Unidentified	(Fruit)				×									0

S_{avg}: average number of *Merosargus* species found per resource type; *Plant resources used only by other Sarginae genera.

for *Merosargus* in the study area. However, this modified index underestimates specificity values compared to the original one.

We also used a measure of host specificity, calculated as

$$\mathrm{MH} = 1 - H, \tag{2}$$

because higher specificity values should be attributed to more specialist species. Conversely, MH represents the proportion of hosts not used by a particular species, among all hosts available minus one.

The overlap in resource use was calculated using the Jaccard similarity index (J). We then constructed a cluster using the Jaccard distance $(1 - J)$ as a measure of distance and the UPGMA amalgamation rule [32].

The Pearson correlation test was used [32] to test if the number of resource types used by each species was correlated with (1) the months when larvae were found, (2) the number of resources shared with other species, and (3) the average Jaccard similarity index (J_{avg}) calculated by averaging the similarity in resource use of each *Merosargus* species with all remaining species.

3. Results

A total of 45 samples with *Merosargus* larvae were collected from 15 plant species, including herbs, vines, palms, and trees and 21 resource types including stems and pseudostems, leaves, fruits, flowers, and inflorescences (Table 1).

Most resources with larvae were collected during a few months ($M_{\text{avg}} = 1.76$ months) of the year. The resource most often sampled with larvae was *H. episcopalis* pseudostems (5 months). In addition, inflorescences of the same plant species were collected in three months. *Thoracocarpus bissectus* (Vell) stems were also sampled in three months. Several plant species containing larvae were collected in only one month. August was the month of the highest resource richness

Table 2: *Merosargus* species with respective plant species and substrate types found in samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004.

Merosargus species	Substrates	
	Substrate type	Plant species
M. cf. *arcuatus*	Stem	*T. bissectus*
M. azureus	Stem	*T. bissectus*, *Urera* sp., *E. edulis*, and Cucurbitaceae sp.1
	Pseudostem	*H. aemygdiana*, *H.* x *matenensis*, *H. spathocircinata*, *H. episcopalis*, and *P. maximum*
	Leaf	Amaranthaceae sp.2
M. cf. *bivittatus*	Inflorescence	*H. spathocircinata*
M. cingulatus	Stem	*A. aculeatissimum* and *T. bissectus*
	Pseudostem	*H. episcopalis*
	Leaf	*A. aculeatissimum*
	Inflorescence	*Musa* sp.
M. cf. *coxalis*	Flower	*L. lurida*
M. gowdeyi	Pseudostem	*H. episcopalis*
	Leaf	Amaranthaceae sp.1
M. gracilis	Stem	*A. aculeatissimum* and *Urera* sp.
	Pseudostem	*H. episcopalis*
	Leaf	*A. aculeatissimum*
M. cf. *opaliger*	Stem	*T. bissectus*
M. aff. *pallifrons*	Inflorescence	*Musa* sp., *H. episcopalis*, and *H. x matenensis*
	Pseudostem	*Musa* sp., *H. spathocircinata*, and *H. episcopalis*
	Bored pseudostem	*H. episcopalis*
M. cf. *pictipes*	Leaf	*A. aculeatissimum*
M. cf. *transversus*	Fruit	*Guarea* sp.
M. varicrus	Inflorescence	*H. spathocircinata* and *H. episcopalis*
	Pseudostem	*H. episcopalis*
	Bored pseudostem	*H. episcopalis*
Other Sarginae genera found		
Acrochaeta sp.	Stem	*T. bissectus*
Ptecticus sp.	Fruit	*Spondias* sp. and *J. princeps*
Himantigera sp.	Fruit	*Guarea* sp. and unidentified

sampled with *Merosargus* larvae ($N = 8$), while only in September plant resources with larvae were not sampled (Table 1).

Individual plant species hosted between 0 and 5 *Merosargus* species. A large proportion of plant species or resource types were used by only one *Merosargus* species. On average, only 1.81 *Merosargus* species were found per resource type (Table 1). The resources that were used by the largest number of species were *H. episcopalis* pseudostems ($N = 5$) and *T. bissectus* stems ($N = 4$). In addition, we never found more than two *Merosargus* species in any sample.

Most *Merosargus* species developed in vegetative organs such as stems, pseudostems, and leaves. However, *M. bivittatus* James, *M. coxalis* Lindner, and *M. transversus* McFadden used only reproductive organs (inflorescences, flowers, and fruits). Three species, *M. cingulatus*, *M. varicrus*, and *M.* aff. *pallifrons*, used both vegetative and reproductive organs, the latter represented by *Musa* sp. or *Heliconia* inflorescences. Larvae of two other genera, *Ptecticus* sp. and *Himantigera* sp., also developed solely in reproductive organs of plants. The species *M.* aff. *pallifrons* and *M. varicrus* were also found in *H. episcopalis* pseudostems attacked by a lepidopteran borer (Table 2).

The plant samples resulted in the emergence of 361 Sarginae adults representing 15 species, including 292 individuals from 12 *Merosargus* species. The species with the highest number of adults were *M. azureus* (Enderlein) ($N = 121$) and *M.* aff. *pallifrons* (Curran) ($N = 58$) (Table 3).

The highest number of *Merosargus* species found in one month was five, in April and August (Table 3). In general, larvae of *Merosargus* species were collected during few months ($M_{\text{avg}} = 2.8$). The species found for the highest number of months were *M. azureus* ($N = 7$), *M.* aff. *pallifrons* ($N = 5$), and *M. varicrus* (James) ($N = 5$).

In total, six *Merosargus* species were reared from only one resource type. On average, few plant species ($S_{\text{avg}} = 2.5$) and

Table 3: *Merosargus* species, month of occurrence, number of adults reared in the laboratory, number of plant species and resources types (in brackets), and resource specificity of each species found in samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004.

Species	Month												Adults	Number of plant species (resource types)	Measure of specificity
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec			
M. azureus	×	×	×	×			×	×			×		121	10 (10)	0.36
M. cf. *bivittatus*	×												2	1 (1)	1.00
M. aff. *pallifrons*	×	×	×	×				×					58	4 (7)	0.79
M. varicrus	×			×				×		×	×		18	2 (4)	0.93
M. cingulatus		×		×				×				×	15	4 (5)	0.79
M. gracilis				×				×					9	2 (3)	0.93
M. gowdeyi					×					×			4	2 (2)	0.93
M. cf. *pictipes*						×						×	6	1 (1)	1.00
M. cf. *arcuatus*							×				×		36	1 (1)	1.00
M. cf. *opaliger*							×						4	1 (1)	1.00
M. cf. *transversus*										×			3	1 (1)	1.00
M. cf. *coxalis*												×	16	1 (1)	1.00
Species/month	4	3	2	5	1	1	3	5	0	3	3	3	Total = 292	$P_{\text{avg}} = 2.5$	$\text{MH}_{\text{avg}} = 0.89$
Ptecticus sp.		×		×									7	2 (2)	—
Himantigera sp.				×	×			×		×			50	2 (2)	—
Acrochaeta sp.							×						10	1 (1)	—

P_{avg}: average number of plant species found; MH_{avg}: measure of specificity (proportion of other plants species not used).

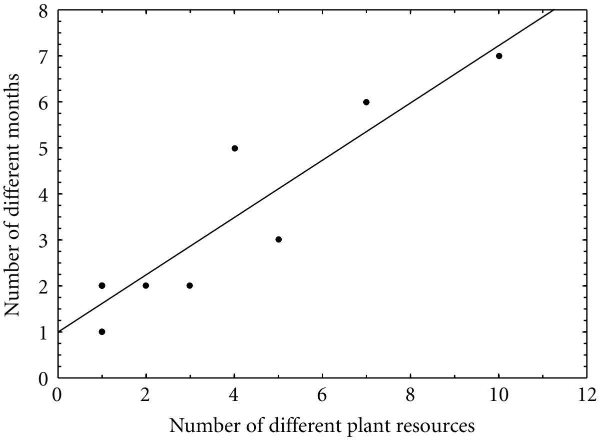

Figure 1: Relationship between the number of plant resource types used by *Merosargus* species and the number of months when larvae were found in samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004.

resource types ($R_{\text{avg}} = 3.1$) were used by each *Merosargus* species. The species that used the greater number of resources were *M. azureus* ($N = 10$), *M.* aff. *pallifrons* ($N = 7$), and *M. cingulatus* Schiner ($N = 5$). In general, species that used more plant resources were also found over a greater number of months ($r = 0.93$; Figure 1).

The average plant species host-specificity measure was $\text{MH}_{\text{avg}} = 0.89$ ($H_{\text{avg}} = 0.11$). This means that a *Merosargus* species feeding on a particular plant species used an average of only 11% of other plant species used minus one.

Although over half of the plant species and resource types were used by a single *Merosargus* species, almost all species shared at least one plant species or resource type with another congener (Figure 2). The exceptions were *M. coxalis* and *M. transversus*, which used a single, exclusive resource type. *Merosargus azureus* used the largest number of exclusive plant species ($N = 5$) and resources types ($N = 6$).

As expected, generalist species were also the ones that shared the most resources with congeners ($r = 0.83, P < 0.05$; Figure 3). However, it should be noted that *M. cingulatus* exhibited greater resource overlap than expected from this correlation.

The small values of the Jaccard similarity index reflected the few overlaps in resource use between *Merosargus* species. The average similarity between a species and the remaining ones was below 25% for all species and below 15% for most species. There was no significant correlation between the number of resource types used and the average similarity indices (Figure 4). Most species exhibited similarity values between 10% and 15%, and this result was observed in more specialized species such as *M. arcuatus* James and *M. opaliger* Lindner as in relatively more generalist species such as *M.* aff. *pallifrons* and *M. azureus*.

The cluster analysis also reflected the small overlap in resource use by *Merosargus* species (Figure 5). The exception was the similarity between *M. arcuatus* and *M. opaliger*: as both used the same single resource their distance was zero in the cluster. Other than these species, *M. pictipes*, *M. gracilis*,

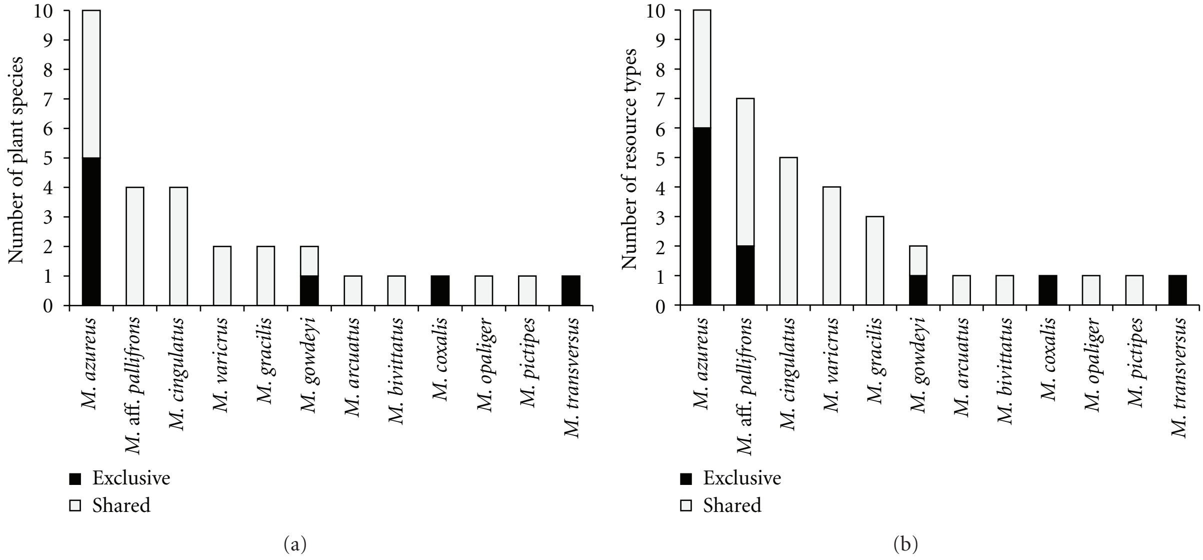

Figure 2: Number of plant species (a) and resource types (b) used by each *Merosargus* species found in samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004. Black bars represent plant species/resource types used by a single species, and open bars those shared with at least one species.

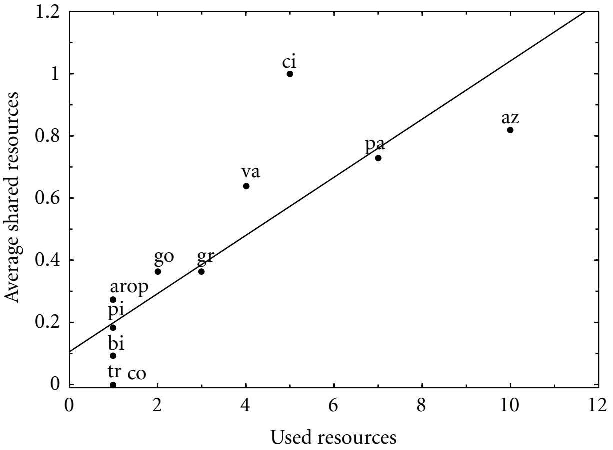

Figure 3: Relationship between the total number of plant resources used by *Merosargus* species and the average number of shared resources found in samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004. Ar-*M. arcuatus*; az-*M. azureus*; bi-*M. bivittatus*; ci-*M. cingulatus*; co-*M. coxalis*; go-*M. gowdeyi*; gr-*M. gracilis*; op-*M. opaliger*; pa-*M. pallifrons*; pi-*M. pictipes*; tr-*M. transversus*; va-*M. varicrus*.

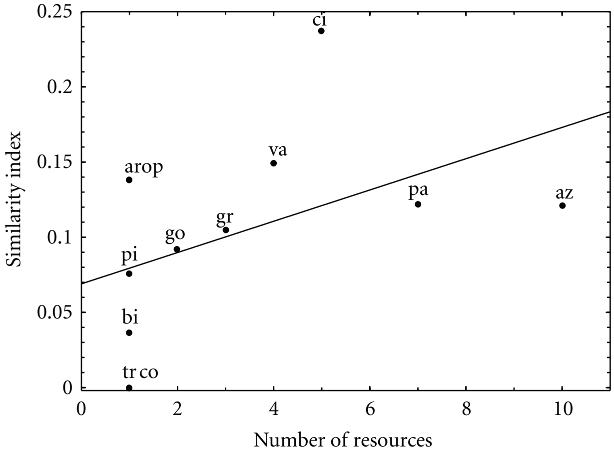

Figure 4: Relationship between the total number of plant resources used by each *Merosargus* species and the average Jaccard similarity index for each species from samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004. ar-*M. arcuatus*; az-*M. azureus*; bi-*M. bivittatus*; ci-*M. cingulatus*; co-*M. coxalis*; go-*M. gowdeyi*; gr-*M. gracilis*; op-*M. opaliger*; pa-*M. pallifrons*; pi-*M. pictipes*; tr-*M. transversus*; va-*M. varicrus*.

and *M. cingulatus* clustered together, and so did *M. pallifrons* and *M. varicrus*. In addition, the two species that used only different, exclusive resources, *M. transversus* and *M. coxalis*, were completely isolated from each other and from all the other species in the cluster.

4. Discussion

In our study we identified several plant species and structures where larvae of a significant number of *Merosargus* species develop. We also showed that *Merosargus* uses a great diversity of substrates for larval development.

All plant species identified here, the genera *Panicum*, *Thoracocarpus*, *Astrocaryum*, *Euterpe*, *Guarea*, *Urera*, and *Musa*, and their respective families, except Arecaceae, shown here to be used by *Merosargus*, are new records. The plants of families Amaranthaceae and Cucurbitaceae whose genera were not identified are also new records for *Merosargus*. In fact, *Merosargus* larvae had only been previously observed

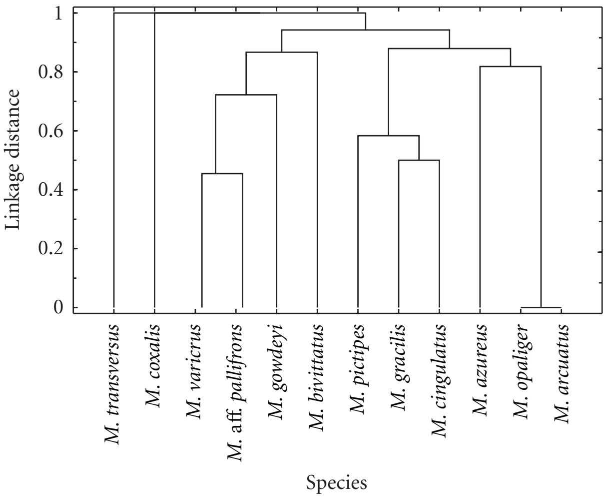

Figure 5: Cluster analysis of *Merosargus* species based on the similarity in resource use by the larvae from samples collected at the Parque Estadual do Rio Doce (PERD), Minas Gerais, Brazil, between October 2001 and October 2004. The cluster was constructed using the Jaccard distance $(1-J)$ and the UPGMA amalgamation rule.

Figure 6: Male of *Merosargus* cf. *varicrus* on the *Heliconia episcopalis* pseudostem with rotten sheath.

in bracts of *Heliconia* (Heliconiaceae) inflorescences [14–16] and fallen flowers of *Couratari stellata* Smith (Lecythidaceae) [17]. Moreover, *Merosargus gracilis* had been recorded in another Arecaceae species (*Bactris gasipaes* Kunth) in the pulp of fruits [18], and not using the trunk or leaves as observed here for the two species of this family: *E. edulis* and *A. aculeatissimum*.

Oviposition occurs on substrates when they are recently damaged or already decaying. The predominance of herbaceous species as a resource for *Merosargus* could be explained by their soft tissues, which are more likely to be found damaged and more suitable for larval feeding. Damage to understory plants is especially likely to occur in the rainy season, when strong winds cause many trees to fall (Fontenelle pers. obs.). In addition, several animals can also cause plant damages. On several occasions, *M. azureus* males were observed approaching and establishing territories on damaged *H. episcopalis* pseudostems immediately after agoutis had gnawed on them (Fontenelle pers. obs.). In fact, some gnawed pseudostems were sampled and yielded several adults of this species.

At the forest fragment in the vicinity of PERD, however, the vertebrate fauna is scarce and there may be less damage to the plants and less availability of vegetative resources for Merosargus. In contrast, may be less frugivory and a large amount of rotting fruit is found on the forest floor, which could be exploited by fly species that use fruit as resources.

Our data reinforced the strong association between *Merosargus* and *Heliconia*. There are five native *Heliconia* species at PERD: *H. episcopalis*, *H. spathocircinata* (Aristeg.), *H. aemygdiana*, *H. angusta* (Vell.) [33], and *H. x matenensis* (Silva et al.) (*H. episcopalis* and *H. spathocircinata* hybrid). Samples from only one native *Heliconia* species did not result in the emergence of *Merosargus* adults (data not shown). There are also two exotic *Heliconia* species at PERD: *H. rostrata* (Ruiz & Pavón) and *H. psittacorum* (L.f.), but they were not sampled. Nevertheless, all *Heliconia* species found at PERD are likely used by *Merosargus* species.

Previous studies have shown *Merosargus* species using only the inflorescences of *Heliconia*. *Merosargus* larvae are part of the phytotelmata fauna of some *Heliconia* inflorescences whose bracts are filled with water and where larvae feed on rotten flowers and other debris [14–16].

Bracts of *Heliconia* species found at PERD do not accumulate large amounts of water. *Heliconia episcopalis*, in particular, has small bracts that remain closed most of the time at an angle that prevents water accumulation, but the inflorescences have dozens of bracts where rotting flowers may be found (Fontenelle pers. obs.). Therefore, despite not collecting water, these inflorescences form an environment rich in rotten organic matter that is somewhat protected from predators and desiccation.

Pseudostems of the *Heliconia* species sampled are likely more important resources for *Merosargus* larvae than inflorescences. Pseudostems can be used in various forms and on several different occasions: when pseudostems of green plants are damaged or attacked by herbivores; when older leaves begin to rot; and when the entire pseudostem is in a more advanced state of decomposition. Bored pseudostems or with rotten sheaths have a strong bad smell. When the borer caterpillar chews on the central pseudostem shaft, it kills the inner leaf, which rots inside the pseudostem. Nevertheless, rotten sheaths are common in older plants and result from normal leaf senescence (Fontenelle pers. obs.).

Two *Merosargus* species, *M.* aff. *pallifrons* and *M. varicrus* (Figure 6), used both bored pseudostems and those with rotten sheaths (data not shown) and they were also found in inflorescences. In all instances larvae were found immersed in liquefied decaying organic matter subjected to heavy hypoxia.

In contrast *M. azureus* had a clear preference for fresh and newly damaged pseudostems (data not shown), which indicates that the volatile compounds that attract this species must be more associated to the plant itself than to its decomposition process.

Couturier et al. [18] found larvae of *Ptecticus* and *Merosargus* in Lecythidaceae flowers. They suggested that flower use is atypical in Stratiomyidae and occurs because of the fetid-scented flowers and the bat floral syndrome of the species of plants used by Stratiomyidae larvae. However, we found *Merosargus coxalis* using the flowers of *Lecythis lurida*, whose flowers are not particularly fetid and are bee-pollinated. Thus, we believe that flower morphology is more important than smell as an explanation for oviposition site selection by *Merosargus*. The morphology of Lecythidaceae flowers is quite distinctive, with stamens arranged in a ring protected by a hood-like, dense cluster of petals [34], forming a wet and safe environment for the larvae. Furthermore, the use of flowers by *Merosargus* larvae is not unusual, as they are very common in *Heliconia* flowers, and is not restricted to a specific floral syndrome, as the *Heliconia* species found at PERD are pollinated by hummingbirds [35].

In fact, several *Merosargus* species are attracted to the smell of rotten plant resources, although this is not true for all species identified in our study. We believe that different species are attracted to different volatile compounds, and the study of these compounds may help elucidate some of the mechanisms responsible for the resource preferences exhibited by different *Merosargus* species.

Additionally, resource preference can have a strong influence on the spatial distribution of populations. Other studies that sampled populations using more standardized methods showed that *Merosargus* species are concentrated in sites where large quantities of preferred resources are found. Sampling with malaise traps in areas with large *H. episcopalis* aggregations resulted in a large number of *M. azureus*, *M. varicrus*, *M. gowdeyi* (Curran), and *M. gracilis* (Williston) adults (Fontenelle et al. unpublished data), which further emphasizes the importance of this plant species as a resource for these insect species.

Because sampling was not uniform little can be said about the distribution of plant resources and species throughout the year. Few plant resources are found in only one season, but resource availability seems to increase during the rainy season.

Therefore, many resources are seasonally available, particularly plant reproductive organs, but vegetative parts may also be more available at certain times of the year. For example, trees fall mostly after the first rains, while borer caterpillars and agouti predation are more common in the dry season (Fontenelle et al. unpublished data).

Merosargus adults are also seasonal. However, more abundant species can be sampled throughout the year (Fontenelle et al. unpublished data), and their larvae and pupae are likely to be even more frequent than adults because the duration of these stages must be greater than that of the adult phase. Moreover, species that use less seasonal resources or a greater variety of resources available at different times could ensure the occurrence of larvae during several months of the year. Therefore, the relationship between the number of resources used and the number of months in which larvae were found should be interpreted with caution. In fact, this pattern may be explained by a correlation between both variables with the species abundance. most abundant *Merosargus* species are likely to be found in more months and may have more known resources.

Merosargus species assemblages in individual plant species were species poor, as they only included up to five species. However, these figures are still higher than those observed for fruit flies (1–3) [31].

The average host specificity of fruit fly species relative to all plant species used as a resource found by Novotny et al. [31] was $H_{\text{avg}} = 0.02$. Using our modified index, the recalculated fruit fly H was $H_{\text{avg}} = 0.03$ for the genus *Bactrocera*, which used 25 plant species (1.67 plant species on average), and $H_{\text{avg}} = 0.13$ for the genus *Euphranta*, which used five plant species (1.50 plant species on average), a more similar result to the one found for *Merosargus* in our study.

We can assume that resource selection for larvae of the genus *Merosargus* is quite specialized. Moreover, we found few substrate types that were used by more than two species, leading to a slight overlap in resource use. Both specialization and little overlap in resource use may promote stable coexistence of *Merosargus* species. In fact, niche differentiation, and particularly resource partitioning, may promote the coexistence of closely related species (e.g., [36]).

Specialization in resource use is a possible explanation for the large number of closely related insect species found in tropical forests. However, some insects groups and guilds are predominantly generalists [21].

Most scavengers such as litter arthropods are often assumed to be generalists because they harvest nutrients from dead plant material and litter-decomposing microbes rather than directly interacting with living plants [22]. However, feeding niche differentiation was demonstrated and considered responsible for the high species richness and diversity of soldierless termites in neotropical rainforests [37].

When competition between members of different species occurs extreme conflicts are expected and the less successful competitor will either be driven to extinction or, more commonly, be forced to modify its feeding pattern [38]. Interspecific interactions among *Merosargus* adults include direct interference in resource use through territorial defense and monopolization (Fontenelle et al. unpublished data). Therefore, resource partitioning may be even more important in this genus as it is so diverse locally, even though males have such intense territorial behavior. Other mechanisms of resource partitioning may occur for species that were clustered together in the cluster analysis such as slight differences in adult activity time or area [38].

Genner et al. [39] studying territorial cichlid fish suggest that ecological generalism reduces the intensity of interspecific competition while specializations for the same resources increase it. We found that one of the most aggressive species *M. azureus* (Fontenelle et al. unpublished data) is the most generalist in resource use. Further studies on *Merosargus* territorial behavior, measuring the intensity of aggression intra- and interspecifically, and degree of resource monopolization will be essential to better understand the selective pressures leading to resource selection of this genus.

Considering the small sample size, the large number of single records, and especially the high plant diversity in forests of the study area, (over 1100 species) ([33, 40, 41]; see also [42] for a phytosociological study), we believe that only a small fraction of the potential resources available to *Merosargus* flies was sampled in this study.

Expanding the sampling effort to new plant species may reduce resource specialization estimates when additional resources are included. Conversely, it could also increase the estimates if additional *Merosargus* species with restricted resource ranges are included.

A taxonomic revision of the group, particularly using molecular techniques, may reveal host-specific cryptic species eventually masking the specialization as has occurred with other groups in Diptera [43, 44]. In addition, it remains to be studied if different genotypes, within a *Merosargus* species, prefer different resource types, an evidence of assortative mating that can act either increasing diversity, through sympatric speciation [26], as reducing the niche breadth [45].

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Research Article

Neutral Sterols of Cephalic Glands of Stingless Bees and Their Correlation with Sterols from Pollen

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Sterols are essential molecules in the membrane lipid composition and precursors of important sterol hormones that regulate many developmental processes. Insects are unable to synthesize sterols *de novo* and, thus, all phytophagous insects depend on an exogenous source of sterols for growth, development, and reproduction. The sterol requirements of social bees are not fully known due to the fact that there is no well-defined diet available throughout the year with regard to floral resources. Our study aimed to characterize the sterols present in pollen stored in *Melipona marginata* and *Melipona scutellaris* colonies, as well as evaluating their presence in the mandibular, hypopharyngeal, and cephalic salivary gland secretions. We analyzed the chemical composition of pollen stored in the colonies and the composition of the cephalic glands of workers in three adult functional phases (newly emerged, nurses, and foragers) by gas chromatography and mass spectrometry. The results showed that the pollen analyzed contained campesterol, stigmasterol, sitosterol, isofucosterol, lanosterol, and small amounts of cholesterol. The glands showed the same compounds found in the pollen analyzed, except lanosterol that was not found in *M. scutellaris* glands. Surprisingly, cholesterol was found in some glands with relative ratios greater than those found in pollen.

1. Introduction

Sterols are essential molecules in cell membrane, they are precursors of sterol hormones and other important regulators of developmental processes. However, insects are unable to synthesize them and have to purchase sterols from their diet [1]. The dietary needs for sterols in insects were firstly reported by Hobson [2] in the blowfly *Lucilia sericata*. Moreover, in 1959, Clark and Bloch [3] showed the inability of insects to synthesize sterols *de novo*, so that they need to purchase these compounds for auxotrophy (from the Greek "to increase" and "nutrition"). Auxotrophic individuals are unable to synthesize organic compounds necessary for its growth. These authors showed the importance of sterols as structural (cholestanol) and metabolic (cholesterol) components in insects. Further studies showed that ecdysteroids derivatives from cholesterol participate in the control of insect growth and maturation [4].

Furthermore, the necessity of sterol ingestion by insects has been demonstrated in different orders including Orthoptera, Hemiptera, Diptera, Lepidoptera, and Hymenoptera [5]. Indeed, all phytophagous insects studied to date rely on exogenous sterol for growth, reproduction and development [6, 7]. Many phytophagous insects are able to convert phytosterols in cholesterol through the dealkylation of compounds containing 28 to 29 carbon atoms, such as desmosterol, campesterol, brassicasterol, fucosterol, and sitosterol [8–10]. However, honeybees are unable to perform this conversion [11, 12]. Other phytophagous insects that donot have the mechanism of dealkylation of phytosterols include two species of Hemiptera (*Oncopeltus fasciatus*, [13]; *Dysdercus fasciatus*, [14]) and one species of Coleoptera (*Trogoderma granarium*, [15]). The ability to convert phytosterols in cholesterol was not verified in other groups of bees.

The sterol requirements of bees are not assessable because their diet and pollen sources remain unknown. The main sterol present in the pollen of plants is 24-methylenecholesterol [16–18]. However, other studies reported that besides the 24-methylenecholesterol, the pollen presents phytosterols as desmosterol, campesterol, fucosterol, sitosterol, and even small quantities of cholesterol [17].

Steroidal compounds might be incorporated and stored in different organs of a bee. Queens and workers of honey bees have a significant amount of 24-methylenecholesterol in their body [19]. Svoboda et al. [1] showed that the 24-methylenecholesterol, isofuscosterol, sitosterol, and campesterol are present in hypopharyngeal and in mandibular glands of honeybee nurses. The size of cephalic glands (mandibular, hypopharyngeal, and labial) in eusocial bees vary according to the age of individuals [20]. In *Apis mellifera*, the mandibular and the hypopharyngeal glands reach a higher peak of development in nurse bees and they are related with food supply to larvae [20]. We hypothesized that, like in *Apis mellifera*, the cephalic glands of stingless bees play a role as storage organs of phytosterols. Our study aimed to characterize the sterols present in pollen stored in *Melipona marginata* and *M. scutellaris* colonies, as well as to evaluate their presence in the mandibular, hypopharyngeal, and cephalic salivary gland secretions.

2. Material and Methods

We used two colonies of Brazilian stingless bee species: *Melipona marginata* and *Melipona scutellaris*. The colonies were kept at the meliponary of Universidade de São Paulo, Ribeirão Preto. For each species, we collected 10 newly emerged workers, 10 nursing workers, 10 foragers, and two samples (1 g) of pollen storage in pots, all on the same day. We marked the new cells produced in the comb that day for later collection of newly emerged workers. The mandibular (MAN), hypopharyngeal (HYP), and labial glands (LAB) (cephalic section) were dissected under stereomicroscope and put individually in 30 μL of hexane. The pollen samples were put in methanol (4 mL/1 g) and the extracts were centrifuged (for removal the proteins). The aqueous phase was removed and dried under N_2 and resuspended in 400 mL of hexane. The samples were injected in a combined gas chromatography-mass spectrometry (GC-MS: SHIMADZU, model QP2010). Separation was achieved on a DB-5MS column 30 m, and the gas carrier was helium at 1.0 mL min^{-1}. The oven temperature was initially set to 50°C (held for 1 min) and increased by 10°C min^{-1} until it reached 300°C (held for 15 min). Analyses were performed in splitless mode. The mass spectra were obtained by 70 eV ionization. The data were analyzed with GCMS solutions for Windows (Shimadzu Corporation), and the chemical compounds were identified based on their mass spectra by comparison with Nist Library data and with a standard solution of different synthetic phytosterols (Sigma-Aldrich).

We analyzed the pollen as described in Silva et al. [21]. The protein fractions of pollen were kept in alcohol 70% and after 24 hours the alcohol was discarded. The samples were placed in vials containing 4 mL of absolute glacial acetic acid (24 hours) according to the acetolysis protocol described by Erdtman [22]. Following acetolysis, the pollen was placed on slides for light microscopy with 50% glycerin. We identified pollen types by comparison with pollen grains deposited on Palinoteca of Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, using a Leica DM 4000B microscope, with an increase of up to 2.560x.

3. Results

Results of sterol analyses of pollen, hypopharyngeal glands (HYP), labial glands (LAB), and mandibular glands (MAN) of *Melipona marginata* and *Melipona scutellaris* are summarized in Tables 1 and 2. The pollen samples collected in pots of the two *Melipona* species showed the same ratios of cholesterol, campesterol, stigmasterol, sitosterol, isofucosterol, and lanosterol. In general, the gland secretions showed the same sterols as pollen, with some variations according to the age of workers and the gland type. The only exception was the absence of lanosterol in the glands of *M. scutellaris* bees, even though it is present in pollen collected in pots. In *M. marginata*, sitosterol was the main sterol present in pollen and glands (except for the mandibular glands of nurse bees) while in *M. scutellaris* campesterol and sitosterol had the highest proportions. Cholesterol in pollen samples had lower relative percentage (0.53% and 0.33%) than others sterols. There was no pattern of increasing or decreasing of the relative percentages of phytosterols present in glands in relation to the age of individuals. The sterols were obtained from Angiosperms as Arecaceae (*M. scutellaris*, 1 species), Fabaceae (*M. marginata*, 4 species; *M. scutellaris*, 3 species), Melastomataceae (*M. marginata*, 1 species) and Myrtaceae (*M. marginata*, 1 species; *M. scutellaris*, 3 species), according to pollen type (Table 3).

3.1. Newly Emerged Workers. The main sterol found in *M. marginata* glands was sitosterol, followed by cholesterol (HYP and LAB) and isofucosterol (MAN). In *M. scutellaris* glands, the main compounds were sitosterol (HYP and MAN) and campesterol (LAB) followed by isofucoterol (HYP) and cholesterol (MAN). In *M. marginata* workers, stigmasterol was found only in mandibular glands and lanosterol was found in mandibular and hypopharyngeal glands. The hypopharyngeal gland and the labial gland of newly emerged workers showed a higher proportion of cholesterol when compared with the pollen in *M. marginata* (26.62%, 47.7% and 0.53%, resp.). In *M. scutellaris*, all glands of newly emerged workers showed a higher percentage of cholesterol when compared with pollen (HYP = 11.20%, MAN = 19.96%, LAB = 20.79%, and Pollen = 0.33%).

3.2. Nurse Workers. Sitosterol was the main steroidal compound found in the glands of nurse bees, except for mandibular glands of *M. marginata* (stigmasterol) and hypopharyngeal glands in *M. scutellaris* (campesterol). Campesterol was the second major sterol found in this class of workers (HYP and MAN in *M. marginata*, MAN in *M. scutellaris*). Only the labial gland of *M. scutellaris* showed isofucosterol as the second main compound. In *M. marginata*, the glands of

Table 1: Relative percentages (Mean ± standard deviation) of sterols in pollen and glands of *Melipona marginata* newly emerged workers (New), nurse bees (Nur), and foragers (For). N = 10 (glands) and N = 2 (pollen samples, 1 g per sample).

		Hypopharyngeal			Mandibular			Labial		
	Pollen	New	Nur	For	New	Nur	For	New	Nur	For
Cholesterol	0.53 ± 0.1	26.62 ± 24.2	1.31 ± 0.6	—	1.20 ± 0.4	2.60 ± 0.01	—	47.7 ± 4.9	14.79 ± 2.3	—
Campesterol	14.98 ± 4.1	14.75 ± 7.3	30.11 ± 18.8	22.16 ± 2.8	6.18 ± 2.8	29.58 ± 13.9	16.49 ± 4.1	—	13.55 ± 1.9	4.06 ± 5.2
Stigmasterol	2.01 ± 0.7	—	23.08 ± 18.7	—	3.39 ± 3.4	54.41 ± 16.8	—	—	14.07 ± 4.8	34.40 ± 44.3
Sitosterol	52.16 ± 17.9	31.38 ± 23.4	35.92 ± 24.8	65.02 ± 12.1	52.45 ± 21.8	13.37 ± 9.5	69.80 ± 29.4	52.3 ± 2.6	23.26 ± 15.4	54.12 ± 69.7
Isofucosterol	23.60 ± 15.2	14.60 ± 5.2	9.58 ± 16.3	12.82 ± 11.4	31.53 ± 10.5	0.29 ± 0.11	13.70 ± 7.4	—	11.56 ± 15.7	7.42 ± 9.6
Lanosterol	6.73 ± 6.06	12.64 ± 8.6	—	—	5.24 ± 1.2	—	—	—	22.78 ± 12.9	—

TABLE 2: Relative percentages (Mean ± standard deviation) of sterols in pollen and glands of *Melipona scutellaris* newly emerged workers (New), nurse bees (Nur), and foragers (For). N = 10 (glands) and N = 2 (pollen samples, 1 g per sample).

		Hypopharyngeal			Mandibular			Labial		
	Pollen	New	Nur	For	New	Nur	For	New	Nur	For
Cholesterol	0.33 ± 0.35	11.20 ± 1.9	2.31 ± 0.8	—	19.96 ± 28.5	21.45 ± 24.0	28.43 ± 11.0	20.79 ± 13.8	14.30 ± 7.5	28.10 ± 36.5
Campesterol	10.97 ± 1.58	5.06 ± 2.1	52.32 ± 31.9	52.71 ± 25.5	16.39 ± 6.7	29.19 ± 11.6	26.88 ± 11.8	31.52 ± 33.9	—	9.64 ± 8.1
Stigmasterol	1.42 ± 1.54	—	—	—	—	—	—	—	—	5.50 ± 8.42
Sitosterol	53.08 ± 19.2	71.27 ± 5.6	34.61 ± 21.8	37.22 ± 37.1	48.18 ± 16.1	34.68 ± 16.9	34.65 ± 28.7	25.90 ± 15.7	58.93 ± 19.7	43.91 ± 28.9
Isofucosterol	27.37 ± 9.9	12.47 ± 2.7	10.76 ± 4.8	10.07 ± 13.4	15.47 ± 3.5	14.69 ± 6.4	10.04 ± 10.1	21.79 ± 24.4	26.77 ± 9.1	12.85 ± 7.2
Lanosterol	6.85 ± 5.9	—	—	—	—	—	—	—	—	—

Table 3: Pollen types found in food pots of *Melipona marginata* and *Melipona scutellaris* colonies.

Families/species	*M. marginata*	*M. scutellaris*
Arecaceae		
Archontophoenix alexandrae (F. Muell.) H. Wendl. and Drude	−	+
Fabaceae		
Anadenanthera macrocarpa (Benth.) Brenan	+	+
Anadenanthera peregrina (L.) Speg.	+	+
Leucaena leucocephala (Lam.) de Wit	−	+
Mimosa sp.	+	−
Senna macranthera (DC. ex Collad.) H. S. Irwin and Barneby	+	−
Melastomataceae		
Tibouchina granulosa (Desr.) Cogn.	+	−
Myrtaceae		
Eucalyptus grandis W. Mill ex Maiden	+	−
Eucalyptus moluccana Roxb.	−	+
Eugenia involucrata DC.	−	+
Eugenia pyriformis Cambess.	−	+

Presence: (+) and absence: (−).

nurse bees showed a higher proportion of stigmasterol when compared with pollen (HYP = 23.08, MAN = 54.41%, LAB = 14.07%, and Pollen = 2.01%). In *M. scutellaris*, all glands of nurse bees showed a higher relative proportion of cholesterol when compared with pollen (HYP = 2.31%, MAN = 20.43%, LAB = 14.30%, and Pollen = 0.33%).

3.3. Forager Workers. Cholesterol and lanosterol were absent in *M. marginata* forager workers. Stigmasterol was present only in labial glands of the two analyzed species. *M. scutellaris* also lacked lanosterol, and sitosterol was the main compound found in the glands, except for hypopharyngeal glands (campesterol). *M. marginata* forager workers showed great relative amounts of campesterol and cholesterol in HYP and MAN gland secretions, and *M. scutellaris* workers presented higher relative concentrations of these same compounds in MAN and LAB glands secretions. In *M. marginata*, the glands of foragers showed a higher proportion of stigmasterol when compared with pollen (LAB = 34.40% and Pollen = 2.01%). Mandibular glands and labial glands of *M. scutellaris* foragers showed a higher percentage of cholesterol when compared with pollen (MAN = 28.43%, LAB = 28.10%, and Pollen = 0.33%).

4. Discussion

This study shows that once sterols are ingested they are distributed among different glands of two stingless bee species. As phytosterols play an important role in sterol metabolism and ecdysteroid production [8, 10, 23–26], the ingestion of exogenous sterols is essential for structural and metabolic components. Sterols seem to be absorbed mainly in the midgut, particularly in phytophagous species [27, 28], moving quickly through mucosal cells [28].

The analyzed pollen and glands lacked 24-methylenecholesterol, differently from results found for *Apis mellifera* by Svoboda et al. [1]. The pollen samples collected in pots of the two *Melipona* species showed the same ratios of sterols despite the differences in pollen origin. Patterson [29] showed that the most abundant sterols found in Angiosperms were sitosterol and stigmasterol, but many species also contain campesterol and dihydrobrassicasterol. The pollen of most plant species accumulate intermediates such as 24-methylenecholesterol and 9β,19-cyclopropyl sterols [30]. However, sitosterol can be the main sterol in some species, as found in *Baccharis viminea*, *Juniperus uthaensis*, *Polygonum* sp., *Hydrophyllum capitatum*, *Pinus sylvestris*, and other plants [31]. Large amounts of stigmasterol and cholesterol were found in the pollen of heather (*Calluna vulgaris*) and in cottonwood (*Populus fremontii*), respectively.

Among bees, 24-methylenecholesterol is the main sterol available in natural diet and the dominant tissue sterol, followed by other phytosterols according to the bee's host-plants [32]. We showed in our study that the main sterols of pollen were present in glands. Moreover, despite the inability of some stingless bees of dealkylation of phytosterols into cholesterol, we found large amounts of cholesterol in some glands. Indeed, the Apocrita (Hymenoptera) lost the dealkylation ability [32], and bees use several unmetabolized sterols. Thus, the accumulation of cholesterol in their glands remains unexplained.

One of the most probable hypotheses to explain the tissue accumulation of sterols is the selective transfer of sterols from the adults' endogenous pools to the offspring [1]. Svoboda et al. [1] showed that sterols of the workers could be transferred to the developing larvae through the brood food material secreted from the hypopharyngeal and mandibular glands and/or the honey stomach of the workers. Tissue sterol profiles can differ between males and females. Female's reproductive organs have higher concentration of cholesterol in *Eurycotis floridana* [33] and queen ovaries of *Apis mellifera* have higher concentrations of 24-methylenecholesterol, campesterol, and sitosterol [34]. Regarding development, sterol profile is influenced by the stage of development (egg, larva and adult) in ants, and the eggs presented the lowest diversity in types of sterol, while the queen had the highest [35].

Relative concentrations in secretions of workers of the same group have high deviations, but this can be explained by the differences in the age of the worker performing a task in colonies of social bees. Ferreira-Caliman et al. [36] showed that engagement in tasks on brood comb (nurse bees) occurred between 5th and 12th days postemergence and the foraging activities initiated after 22 days postemergence and this task can be observed at least for 30 days.

This study is the first report on the participation of cephalic glands as storage tissues of pollen phytosterols in stingless bees. We could not assess how the accumulation of phytosterols occurs throughout life of the individual, because no bioassay using a controlled diet was performed. However, we showed that the glands of the head of stingless bees are an important storage tissues for these compounds, although many studies are still needed to understand how this transfer occurs and the role of these glands as reservoirs of sterols.

Conflict of Interests

The authors declare that there was no conflict of interests or any research financial gain concerning the results of the present study.

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Research Article

Foraging of *Scaptotrigona* aff. *depilis* (Hymenoptera, Apidae) in an Urbanized Area: Seasonality in Resource Availability and Visited Plants

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The floral sources used by bees can be identified by analyzing pollen grains obtained from their bodies, feces, brood cells, or storage pots in the nests. In addition to data on resource availability, this information enables the investigation on the selection of food resource by bees. We assessed the foraging patterns of *Scaptotrigona* aff. *depilis* in an urbanized area with seasonal availability of food resources. The species visited a percentage of 36.60% of the available flora, suggesting that these bees are selective at spatiotemporal scale. When many types of resources were available, the workers concentrated their collection activities on a limited group of sources. In contrast, more plant species were exploited during periods of lower number of flowering plants. A monthly analysis of the foraging patterns of the studied colonies revealed that *Syzygium cumini* (88.86%), *Mimosa* sp.1 (80.23%), *Schinus terebinthifolius* (63.36%), and *Eucalyptus citriodora* (61.75%) were the most frequently used species and are therefore important for maintaining *S.* aff. *depilis* at the study area. These plants are close to the colonies and exhibit mass flowering. This study is one of few works to quantify natural resource availability and to analyze the effects of flowering seasonality on the selection of food sources by bees.

1. Introduction

The Meliponini, popularly known as stingless bees, are highly social organisms that occur in tropical and subtropical areas throughout the world [1]. One of the many important activities performed by workers is the collection of nectar (an important source of carbohydrates) and pollen (a source of protein and vitamins) [2, 3]. These resources are collected and stored in the colonies to feed adult and immature bees. The collection of these resources is primarily regulated by abiotic factors such as environmental temperature, relative humidity, and wind speed [4, 5]. Among the biotic factors that can influence foraging are the morphological and physiological characteristics of the bee species, resource availability, and the reproductive status of the colony [6, 7].

While collecting pollen and/or nectar from flowers, bees usually stay with pollen grains adhered to various parts of the body besides those pollen stored into the corbicula. As a result, on subsequent visits, the bees may unwittingly perform pollination [8]. Bees, along with other animals, pollinate up to 94% of angiosperms in tropical areas [9]. Several species of stingless bees have been considered good candidates as commercial pollinators because they can easily be kept in hives, have numerous workers per colony, and are nonaggressive [10, 11]. In addition to nine crops pollinated by stingless bees and related by Heard [11], studies made after that publication showed that stingless bees are also effective pollinators of other nine crops [12]. Conservation of forest fragments around such crops can provide resources for the bees, maintaining pollinator populations and thereby improving crop pollination rates [13, 14].

Stingless bees, which maintain perennial colonies, visit multiple types of flowers to obtain resources and are thus considered to be generalists [1]. However, they can intensify collection at certain sources, indicating some degree of selectivity [15–19], as also observed in specialized solitary bees [20, 21]. Their focus on a few selected sources can be explained by optimal foraging theory [22], which proposes that bees concentrate their visits on the most profitable sources, that is, those that offer more energy than what spent to acquire the resource.

The food sources exploited by bees can be identified by direct observation of the visiting bee at the flower [23, 24] or by pollen analysis [14, 25, 26]. Pollen analysis enables quantification of the diversity and frequency of pollen grains found on the bodies of the bees [14, 27], the nest [28, 29], and/or feces of adult and immature bees [13].

Studies aiming to identify the food sources used by bees are considered more complete if there is an estimation of resource availability performed throughout the study period [27, 30–32]. However, due to the difficulty of evaluating resource availability under natural conditions, only a few studies were made with this focus [33].

Data on the plant species that bees use as food sources improve our understanding of their food resources and niche overlap between species [27]. Furthermore, this information provides a base for studies on the interactions between the bees and their preferred plants as well as support for the elaboration of management plans and the conservation of both bees and plant species that depend on them for pollination (and, consequently, for reproduction) [14].

This study aimed (i) to identify the floral sources used by *Scaptotrigona* aff. *depilis* by analyzing the pollen loads from returning workers and (ii) to assess the foraging pattern of this species in relation to the availability of floral resources.

2. Material and Methods

2.1. Study Area and Bee Species. The study was performed in the experimental meliponary of the Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo (USP) (21°10′30 S and 47°48′38 W). The University campus is urbanized but retains areas with plants that are native to seasonal semi-deciduous forests as well as exotic plants used in urban landscape projects. There is also a 75 ha forest area planted with species that are typical of the original vegetation [34]. The local climate is characterized by two well-defined seasons: a cool/dry season extending from May to September and a hot/wet season extending from October to April.

In Brazil, *Scaptotrigona* aff. *depilis* is found at Rio Grande do Sul, Paraná, Mato Grosso do Sul, São Paulo, and Minas Gerais states [35]. The term “affinis” is used when the identity of a distinct biological species is unknown but it has a strong similarity with a known species, in this case *Scaptotrigona depilis* (Moure, 1942). Nests of *S.* aff. *depilis* are found in tree cavities, its colonies are populous, and the workers are very aggressive defending the nest by flying around and biting invaders. For this study, four strong colonies with similar population sizes and distant from each other by up to 15 m were selected.

2.2. Spatiotemporal Distribution of Floral Resources. The spatiotemporal distribution of floral sources was evaluated from an area of 500 m radius from the place where the nests of *S.* aff. *depilis* were maintained. Monthly surveys were conducted within this area from March 2010 to February 2011, and species of flowering plants were identified across the vertical strata (trees, shrubs, herbaceous plants, and vines) [14]. The surveys were made during five days per month, totaling 480 hours along the year. The number of individual plants in flower was used to assess the availability of resources. Samples were collected from flowering plants and deposited in the Herbário SPFR (Herbarium of the Departamento de Biologia-FFCLRP-USP). Flower buds of the same species were also collected, the pollen grains were removed and, after acetolysis, mounted on reference slides, which were subsequently deposited in the palynotheca of the Laboratory of Pollen Ecology of FFCLRP-USP.

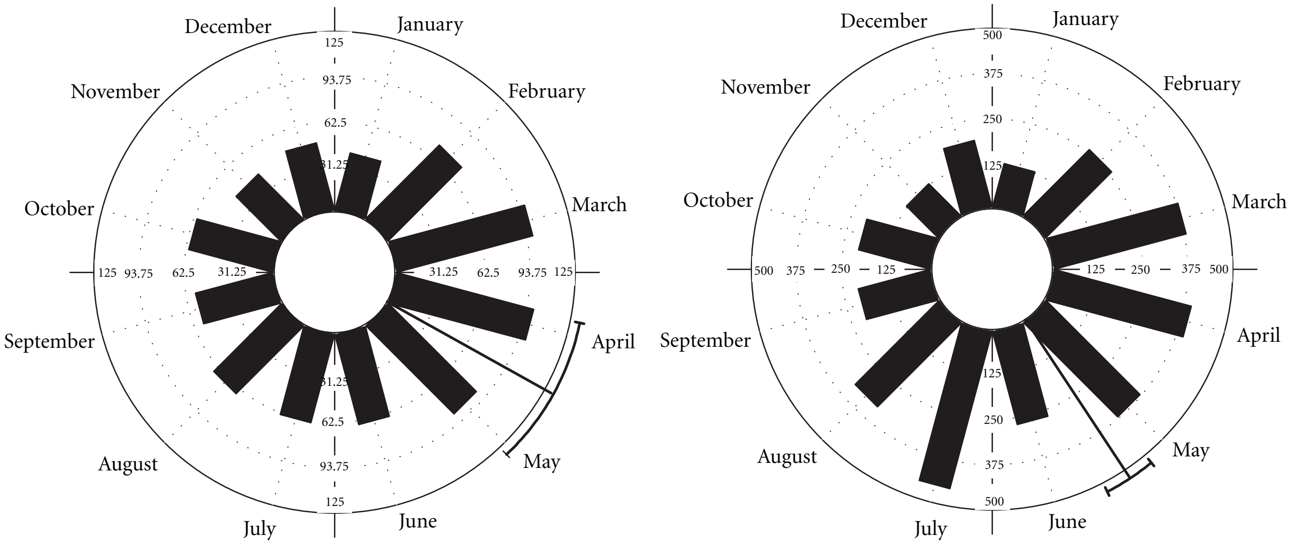
2.3. Pollen Samples. To assess pollen loads content, 40 returning foraging workers were collected monthly from each colony ($n = 4$) for a total of 160 samples per month. Collections were performed between 7:30 am and 1:30 pm, depending on climatic conditions and pollen foraging activity. This activity was evaluated by counting the number of workers who returned to the colony with pollen load, through observation during the morning (5-minute scans at 30-minute intervals) and afternoon (5-minute scans at 60-minute intervals) one day per month. This method was adopted because pollen collection by workers is known to diminish considerably in the afternoon [36, 37].

Corbicula pollen loads were removed using pincers, placed in Falcon tubes containing 2 ml of 70% ethanol and fixed for at least 24 h [14]. The tubes were then centrifuged for 15 min, and the ethanol was discarded. Glacial acetic acid (4 mL) was added to the remaining pollen material, which was then acetolysed [38] and placed on slides prepared with Kisser gelatin and deposited in the palynotheca at FFCLRP-USP. The pollen grains were identified by comparison with other materials deposited in the palynotheca.

2.4. Data Analysis. Pearson’s correlation coefficient (r) [39] was calculated to examine the relationship between the mean number of returning pollen workers from the four colonies and the temperature, relative air humidity, and wind speed. This coefficient was also used to examine the relationship between the number of different pollen types collected and the numbers of species and individuals in flower. These analyses were conducted using R (version 2.13.1, R Development Core Team 2009).

The climatic data used in the analysis were obtained from a weather station located next to the meliponary (available at: http://www.b-lab.at/B-Lab-Research/B-Lab-Research.html).

The spatiotemporal distribution of floral resources was evaluated by a circular analysis implemented with Oriana 4.0 [40]. This analysis was performed for both the number of



(a) (b)

Figure 1: Circular analysis of resource availability during one year (March 2010 to February 2011) in the University campus. (a) Number of species in bloom. (b) Number of individuals in bloom. Each month represents 30° of the circle, and each bar represents the number of flowering species and individuals, respectively. The line at the top of the vector indicates the standard deviation.

species and number of individuals in flower in the study area. Circular analysis was also used to evaluate the number of *S.* aff. *depilis* workers performing pollen collection activities.

Analysis of the pollen collected by the bees was performed using a binocular microscope with up to 2560x magnification. Digital images of the pollen grains were taken using a camera attached to the microscope. Qualitative analysis was used to assess the richness of the visited species and was supplemented by a quantitative analysis of the first 400 pollen grains from each sample [41]. The monthly percentages and the following occurrence classes were then determined: dominant pollen (>45% of the total number of grains on the slide), supplementary pollen (15 to 44%), important isolated pollen (3 to 14%), and occasionally isolated pollen (<3%) [42–44].

An analysis of the Sørensen similarity coefficients using UPGMA clustering was made to examine the overlap in resource use between the four colonies. These analyses were conducted using R (version 2.13.1, R Development Core Team 2009).

3. Results and Discussion

A total of 3285 individuals were distributed in 235 species and flowered in the studied area. The mean date of flowering species was significant ($z = 20.60$; $P < 0.001$), with a concentration of species occurring around April ($r = 0.16$) (Figure 1(a)). The highest numbers of flowering species were observed during March and April 2010, with 97 and 98 species, respectively. The mean date was also significant for the flowering individuals ($z = 167.50$; $P < 0.001$) with a concentration of them in May ($r = 0.23$). The majority of the plant individuals flowered in July ($n = 450$) (Figure 1(b)).

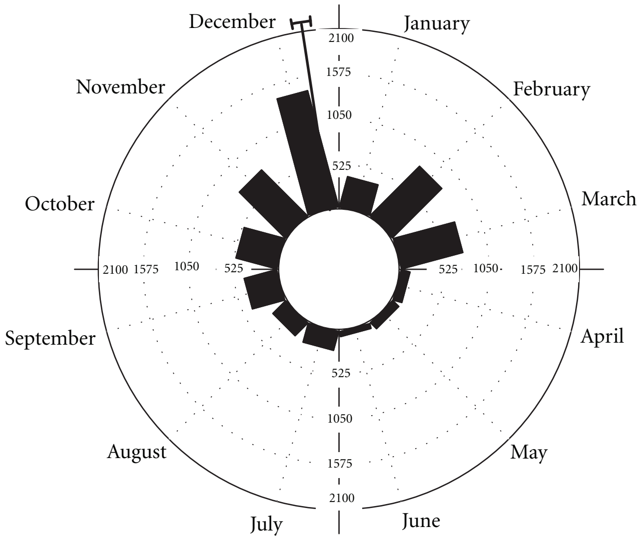


Figure 2: Circular analysis of the mean number of pollen foraging workers of *Scaptotrigona* aff. *depilis*. Each month represents 30° of the circle, and each bar represents the number of returning pollen workers. The line at the top of the vector indicates the standard deviation.

The number of returning pollen workers differed across the study period, with a significant peak in December ($z = 1171.24$; $P < 0.001$), revealing an accentuated seasonal pattern ($r = 0.45$) (Figure 2). Out of all the climatic variables evaluated on the collection days, only temperature was positively correlated with the number of workers returning with pollen loads (Table 1). This result could explain the lower mean number of workers returning with pollen loads observed in June, as this collection day had the

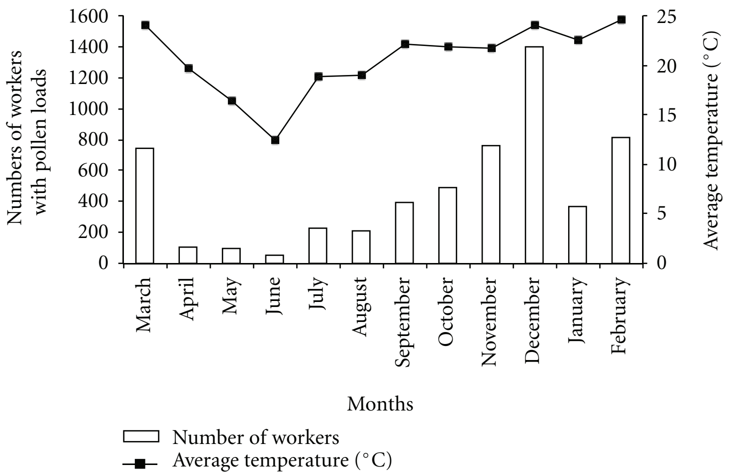


Figure 3: Mean number of pollen foraging workers of *Scaptotrigona* aff. *depilis* related to the average temperature.

Table 1: Pearson coefficients (*r*) between the mean number of returning foraging workers and climatic factors and between pollen collected and resource availability.

	r	*P*
Mean number of returning pollen workers × mean temperature	0,754	0,00464
Mean number of returning pollen workers × relative air humidity	0,174	0,5888 (ns)
Mean number of returning pollen workers × wind speed	−0,012	0,9713 (ns)
Pollen types × number of flowering species	−0,479	0,1154 (ns)
Pollen types × number of flowering individuals	−0,346	0,2698 (ns)

*ns: not significant.

lowest mean temperature (16.6°C) recorded over the year (Figure 3). The reduced number of workers observed in June was apparent when the colonies were analyzed separately (Figure 4). Temperature is an important determining factor for the normal activity of bees, being perhaps the most important factor for regulating the onset of flight [45–48]. Low temperatures reduce metabolic rates, thereby impeding flight activity and other movements in bees [49].

Eighty-six pollen types belonging to 66 genera in 36 botanical families were identified in the samples. From this total, 83.72% were identified to the species level, while the others were identified to the family or genus level or remained unidentified (Table 2).

The families with the highest number of visited species were Fabaceae, Malvaceae, and Myrtaceae, accounting for 36.04% of the total species visited (Figure 5). Species of Fabaceae and Myrtaceae were considered as important sources of pollen due to frequency of occurrence of different pollen types (dominant, supplementary, or important isolated pollen). Malvaceae, on the other hand, considering the frequency of occurrence was important source of nectar (occasionally isolated pollen), including *Basiloxylon brasiliensis* (Allemão) K. Schum. which was also important as a source of pollen (Table 2). Eusocial bees, such as those of the Meliponini tribe and *Apis mellifera* L. (Apini), were previously reported to most commonly visit Fabaceae and Myrtaceae species [28, 50, 51].

Ferreira et al. [52], who examined pollen loads of workers of *Scaptotrigona depilis* over one year in an urban area in Dourados (Mato Grosso do Sul, Brazil), reported that a total of 42 species were visited and that the Myrtaceae and Fabaceae-Mimosoideae families were the most highly represented. Similarly, Marques-Souza et al. [53], who studied the pollen loads of *Scaptotrigona fulvicutis* (Moure 1964) in an old regrowth forest in Manaus (Amazonas, Brazil), showed that these bees collected pollen from the flowers of 97 plant species distributed across 73 genera and 36 families, with the most frequently visited species belonging to the Fabaceae-Mimosoideae, Myrtaceae and Sapindaceae families.

Ramalho et al. [51] proposed that it is not unexpected that social bees should most frequently visit plant families containing many Neotropical species, such as the Fabaceae, Malvaceae and Myrtaceae, for both pollen and nectar, with possible regional differences. Additionally, species of Myrtaceae commonly hold open flowers with many stamens and anthers opening lengthwise, exposing the pollen grains, and facilitating visits by bees [52].

Notably, of all the plant species visited, *S.* aff. *depilis* concentrated its collection on only a few sources along the year. Based on the monthly percentage, the most frequently visited species were *Syzygium cumini* (L.) Skeels (Myrtaceae) (in October), *Mimosa* sp. 1 (Fabaceae) (in January), *Schinus terebinthifolius* Raddi (Anacardiaceae) (in February), and *Eucalyptus citriodora* Hook. (Myrtaceae) (in May) (Figure 6) once their pollen grains were classified as dominant in the samples (Table 2). *Eucalyptus* species are often cited as commonly used by Meliponini. Ramalho [54] attributes this loyalty to the high concentration of pollen in the crowns of these trees, which serve as a visual display for the bees. This pattern was also observed by Cortopassi-Laurino and Ramalho [28], who studied *Trigona spinipes* (Fabricius, 1793) and *Apis mellifera*.

Table 2: Plant species used by *Scaptotrigona* aff. *depilis*, based on analyses of the pollen load from workers of 4 colonies, between March 2010 and February 2011 in the University campus.

Family	Species	Mar	Apr	May	Jun	Jul	Ago	Sep	Oct	Nov	Dec	Jan	Feb
Acanthaceae	*Hypoestes sanguinolenta* (Van Houtte) Hook. f.					11.67							
Amaranthaceae	*Chamissoa altissima* (Jacq.) Kunth			1.25	5.28	3.50			0.02		0.02		
	sp.1				18.25	3.52	0.02	0.02					
Anacardiaceae	*Anacardium occidentale* L.												0.17
	Myracrodruon urundeuva Allemão					20.92	32.52	8.34	1.80	20.37			
	Schinus terebinthifolius Raddi	5.59		12.83	8.41	0.54		10.92		37.82	12.55	7.92	63.36
	sp.1					3.75							
Arecaceae	*Archontophoenix alexandrae* (F. Muell.) H. Wendl. and Drude						2.27						
	sp.1									0.02			0.16
	Syagrus romanzoffiana (Cham.) Glassman							3.73		2.93	0.05		
Asteraceae	*Bidens sulphurea* (Cav.) Sch. Bip.											0.10	
	Tithonia diversifolia (Hemsl.) A. Gray				0.02	0.02							
	Sphagneticola trilobata (L.) Pruski			0.28									
Bignoniaceae	*Handroanthus roseo-albus* (Ridl.) Mattos							24.05	0.05				
Boraginaceae	*Cordia trichotoma* (Vell.) Arráb. ex Steud.				0.09						0.05		
Cannabaceae	*Celtis iguanaea* (Jacq.) Sarg.									0.10	0.03	0.02	
Caricaceae	*Carica papaya* L.							1.84					1.80
Chrysobalanaceae	*Licania tomentosa* (Benth.) Fritsch					0.04				0.07			
Calophyllaceae	*Calophyllum brasiliense* Cambess.							4.81					
Commelinaceae	*Tradescantia pallida* (Rose) D. R. Hunt					1.35	1.19	0.03					
	Tradescantia zebrina Heynh.							0.05	0.09	0.42			
Dilleniaceae	*Doliocarpus dentatus* (Aubl.) Standl.			2.00									
Euphorbiaceae	*Croton urucurana* Baill.			1.81							1.30	0.10	0.73
	Joannesia princeps Vell.				4.95		4.06	0.05		10.72	0.03		
	Ricinus communis L.	2.72	2.42	0.06	8.91						10.70		0.25
	sp.1				0.28								
Fabaceae	*Anadenanthera macrocarpa* (Benth.) Brenan								2.56	2.77	0.05	1.13	2.86
	Cassia grandis L. f.								1.84	0.33			
	Centrosema sp.					0.04							
	Delonix regia (Bojer ex Hook.) Raf.									0.10	13.34	0.20	0.03
	Erythrina cristae-galli Krukoff		0.17										
	Gliricidia sepium (Jacq.) Kunth ex Walp.							0.02					
	Inga vera Willd							2.17					
	Leucaena leucocephala (Lam.) de Wit	0.61	2.03	13.61	5.17	0.77		0.02	0.91	0.47	14.52	0.33	0.02
	Machaerium aculeatum Raddi		1.39							3.92	1.45		
	Mimosa sp.1	5.89	5.67					0.03		0.02	0.03	80.23	0.02
	Mimosa sp.2	20.78	19.14		5.11								27.22
	Poincianella pluviosa (DC.) L. P. Queiroz.							1.19	0.03	0.10		0.17	
	Senna spectabilis (DC.) H. S. Irwin and Barneby		0.17										
	sp.1		0.55										
	Tipuana tipu (Benth.) Kuntze							0.83				1.98	

Table 2: Continued.

Family	Species	Mar	Apr	May	Jun	Jul	Ago	Sep	Oct	Nov	Dec	Jan	Feb
Heliconiaceae	*Heliconia psittacorum* L. f.							0.11					
Lamiaceae	*Ocimum gratissimum* L.	0.03											
	Tectona grandis L. f.												0.03
Lythraceae	*Lagerstroemia indica* L.										0.38		
	Lagerstroemia speciosa (L.) Pers.										0.63		
Malvaceae	*Basiloxylon brasiliensis* (Allemão) K. Schum.						18.05						
	Bombacopsis glabra (Pasq.) Robyns						0.02						
	Ceiba speciosa (A. St.-Hil.) Ravenna	0.08											
	Guazuma ulmifolia Lam.	1.30						0.02		0.03			
	Heliocarpus americanus L.							1.97					
	Luehea divaricata Mart.					0.02							
	Pseudobombax grandiflorum (Cav.) A. Robyns									0.03			
	Triumfetta rhomboidea Jacq.					19.42	0.52						
Melastomataceae	sp.1		0.09										
	Tibouchina granulosa (Desr.) Cogn.								0.50				
Meliaceae	*Cedrela odorata* L.								0.19	0.03			0.06
Muntingiaceae	*Muntingia calabura* L.	0.31	0.22		1.61	0.06		4.52	0.75	0.48	0.41	0.65	
Myrtaceae	*Eucalyptus citriodora* Hook.	40.64	35.47	61.75	2.72	17.23	3.41	8.00					3.09
	Eucalyptus grandis W. Mill ex Maiden					7.48	15.97						
	Eucalyptus moluccana Roxb.	14.36	0.23		14.55	1.12			0.70	5.13	0.06	7.07	0.20
	Eugenia brasiliensis Lam.									3.77			
	Eugenia involucrata DC.						1.78		0.13				
	Eugenia pyriformis Cambess.							0.02	0.03				
	Eugenia uniflora L.	2.20	11.05		4.50	4.56	0.03	4.33					
	Syzygium cumini (L.) Skeels				16.14	2.37		13.92	88.86	0.27			
Oleaceae	*Ligustrum lucidum* W. T. Aiton			5.81	0.27						42.97	0.03	
Phytolaccaceae	*Gallesia integrifolia* (Spreng.) Harms	5.27	21.33							8.23			
Poaceae	*Paspalum notatum* Alain ex Flüggé	0.02	0.02	0.11	0.03			0.02					
Polygalaceae	*Antigonon leptopus* Hook. and Arn.		0.02										
Rubiaceae	*Ixora chinensis* Lam.				3.72						1.42		
	sp.1										0.02		
Rutaceae	*Citrus latifolia* (Tanaka ex Yu.Tanaka) Tanaka							0.41				0.07	
	Citrus limonia Osbeck						0.02			0.05			
Salicaceae	*Casearia sylvestris* Sw.						0.73						
Sapindaceae	*Paullinia elegans* Cambess.	0.11	0.03										
	Serjania lethalis A. St.-Hil.					0.67	19.44	4.25					
Solanaceae	*Acnistus arborescens* (L.) Schltdl.									0.03			
	Brunfelsia uniflora (Pohl) D. Don							4.27					

Table 2: Continued.

Family	Species	Mar	Apr	May	Jun	Jul	Ago	Sep	Oct	Nov	Dec	Jan	Feb
Strelitziaceae	*Ravenala madagascariensis* Sonn.			0.50									
Urticaceae	*Cecropia pachystachya* Trécul	0.09						0.02					
Verbenaceae	*Duranta erecta* L.					0.90				0.10			
Zingiberaceae	sp.1									0.22			
Unidentified	Unidentified 1					0.06		0.08	1.55				
	Unidentified 2									0.33			
	Unidentified 3									1.17	0.02		

*Data are presented as monthly percentages (%). Dominant pollen (>45%), supplementary pollen (15 to 44.99%), important isolated pollen (3 to 14.99%), and occasionally isolated pollen (<3). Mar: March, Apr: April, May: May,…Feb: February.

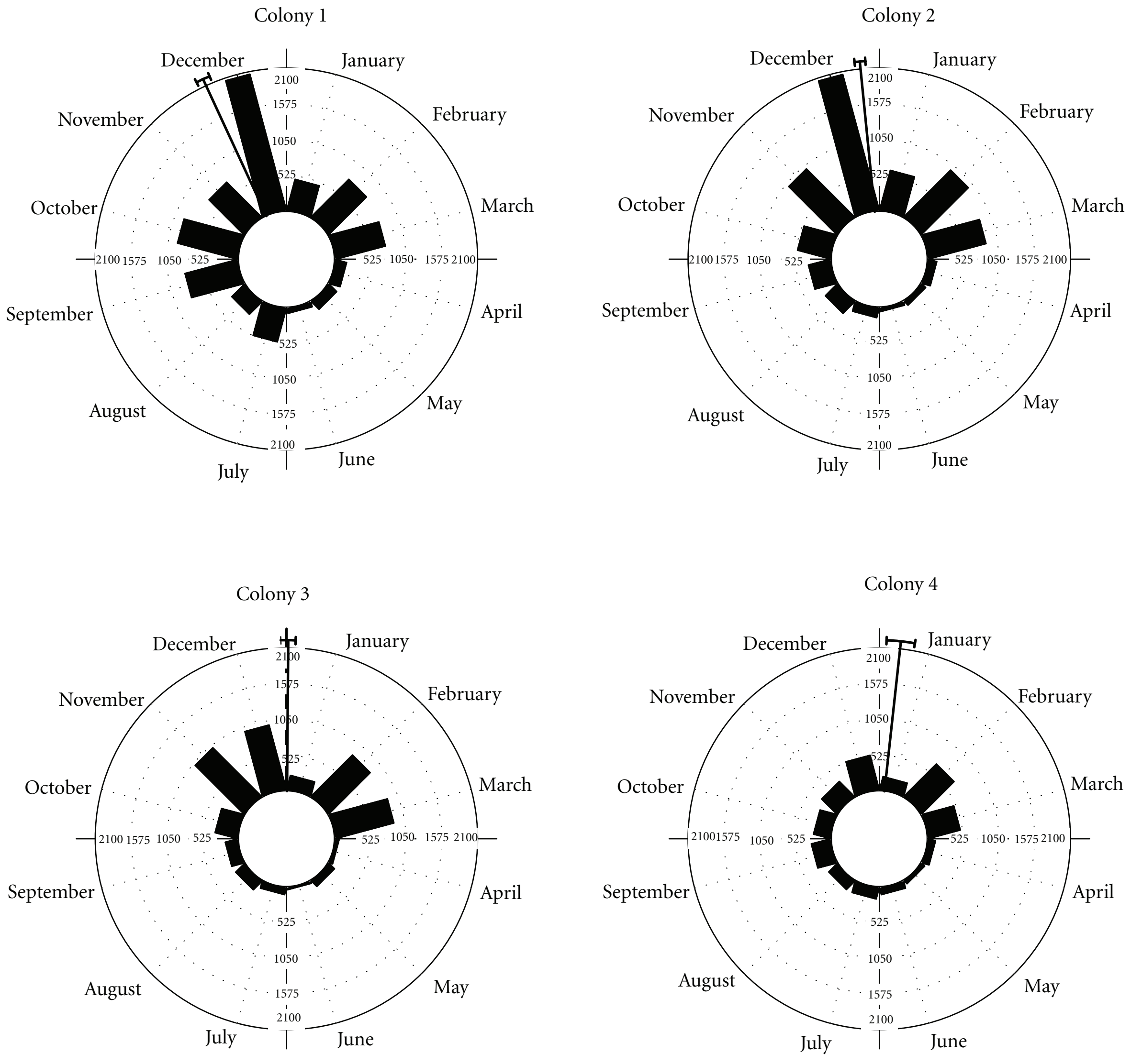


Figure 4: Circular analysis of the number of pollen foraging workers of *Scaptotrigona* aff. *depilis* for each colony. Each month represents 30° of the circle, and each bar represents the number of returning pollen workers. The line at the top of the vector indicates the standard deviation.

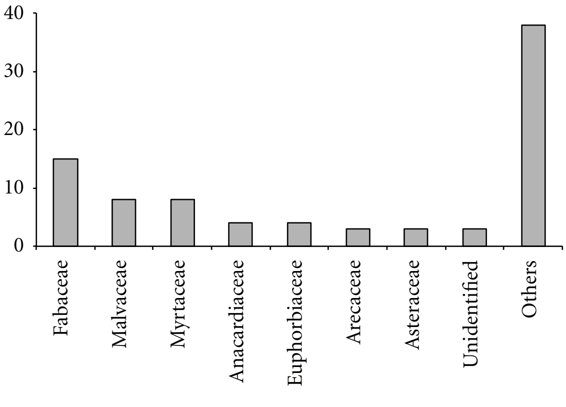
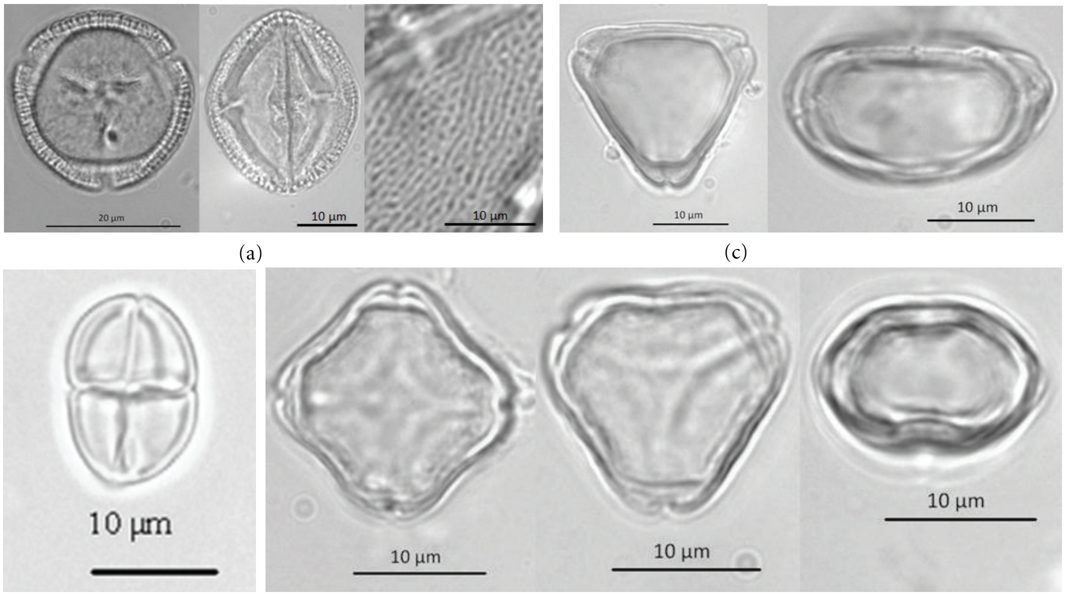


Figure 5: Number of species visited by *Scaptotrigona* aff. *depilis* per plant family during one year (March 2010 to February 2011) in the University campus.

Ricinus communis L., *Leucaena leucocephala* (Lam.) de Wit, *Eucalyptus moluccana* Roxb., *Eugenia uniflora* L., and *Muntingia calabura* L. were also important in the study area because they provided food to the colonies for a period of at least six months (Table 2).

Species of the genus *Scaptotrigona* forage in groups [55] and exhibit recruitment mechanisms, for example, scent trails and source marking [56, 57], that result in the concentration of workers on few sources of pollen. *Scaptotrigona* aff. *depilis* also behaves aggressively and can therefore dominate certain resources [58]. Factors such as proximity to the colony, high protein content, and mass flowering may also explain the high frequency with which certain plants are used by bees [6].

Of the 235 species of flowering plants sampled in the meliponary and its surrounding area, *S.* aff. *depilis*



(a) (c)

(b) (d)

Figure 6: Photomicrographs of pollen grains of the most used species by *Scaptotrigona* aff. *depilis*. (a) *Schinus terebinthifolius* Raddi (Anacardiaceae) in polar and equatorial view and detail of the exine. (b) *Mimosa* sp1 (Fabaceae), a polyad. (c) *Eucalyptus citriodora* Hook. (Myrtaceae) in polar and equatorial view. (d) *Syzygium cumini* (L.) Skeels (Myrtaceae) in polar and equatorial view and detail of the exine.

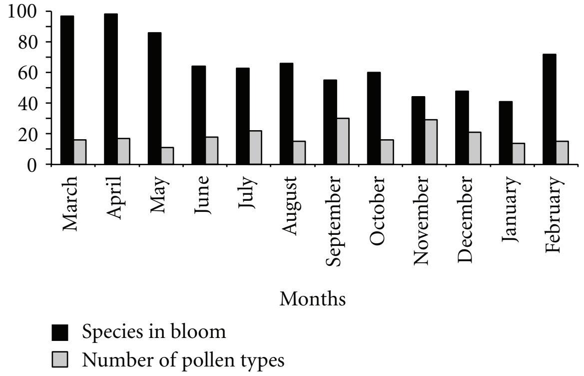


Figure 7: Number of flowering species and species used by *Scaptotrigona* aff. *depilis* during one year (March 2010 to February 2011) in the University campus.

used 36.60% of the available resources. Other stingless bees species also obtain most of their food from a small number of sources [16, 18, 50]. Antonini et al. [59] found that *Melipona quadrifasciata* Lepeletier, 1836, visited only 19.30% of the flowering plants in its habitat. This preference may be related to the fact that social bees, even when classified as generalists, focus their collection activities on the sources that are most profitable and nearest to the colony, as predicted by optimal foraging theory [22, 27].

The number of pollen types collected by bees and the number of species and individuals in flower were not correlated (Table 1). However, an analysis of the ratio between the number of flowering species and species used each month revealed that in November, *S.* aff. *depilis* used 65.91% of the available sources in the study area (Figure 7). Besides those species, two others unidentified species were also used by the bees (Table 2). Certainly, these species were growing out of the study area. Thus, in November, the resource collection radius had been expanded. According to Eltz et al. [27], when there are few resources available in the environment, workers are forced to forage at more distant sources, and the larger the radius of the foraged area, the higher the probability that workers from different colonies will visit different food sources. The reverse was also true, as during the months of March, April, and May, which were periods of greater resource availability, workers harvested proportionately fewer pollen types as compared with other months. In periods with a greater amount of resource availability, the bees could switch from less profitable to more profitable resources, which could lead the colonies to concentrate on particular sources [27].

Cluster analysis showed a high overlap among the colonies, with the distance (Sørensen similarity coefficients) between them being small. The largest distance (0.294) occurred between colonies 1 and 4 (Figure 8). This high overlap of resource use among the colonies was expected, as they belong to the same species and share morphophysiological constraints and possibly the pheromone trails marking the sources. Additionally, as the four colonies were located relatively close together within the meliponary, they share the same foraging area. More detailed studies may show whether intraspecific resource sharing occurs at the same or different times of day, which would allow them to avoid competition.

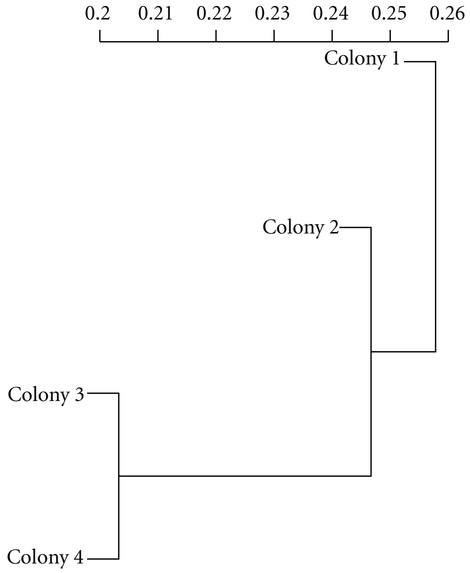


Figure 8: Dendrogram based on qualitative similarity of the pollen collected by 4 colonies in the study period. Distances are Sørensen Index; clustering was done with UPGMA.

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Research Article

Predation of Fruit Fly Larvae *Anastrepha* (Diptera: Tephritidae) by Ants in Grove

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Based on evidence that ants are population regulatory agents, we examined their efficiency in predation of fruit fly larvae *Anastrepha* Schiner, 1868 (Diptera: Tephritidae). Hence, we considered the differences among species of fruit trees, the degree of soil compaction, and the content of soil moisture as variables that would explain predation by ants because these variables affect burying time of larvae. We carried out the experiment in an orchard containing various fruit bearing trees, of which the guava (*Psidium guajava* Linn.), jaboticaba (*Myrciaria jaboticaba* (Vell.) Berg.), and mango trees (*Mangifera indica* Linn.) were chosen for observations of *Anastrepha*. We offered live *Anastrepha* larvae on soil beneath the tree crowns. We observed for 10 min whether ants removed the larvae or the larvae buried themselves. Eight ant species were responsible for removing 1/4 of the larvae offered. The *Pheidole* Westwood, 1839 ants were the most efficient genus, removing 93% of the larvae. In compacted and dry soils, the rate of predation by ants was greater. Therefore, this study showed that ants, along with specific soil characteristics, may be important regulators of fruit fly populations and contribute to natural pest control in orchards.

1. Introduction

The fruit fly *Anastrepha* spp., together with some rarer *Rhagoletis* Loew, 1862, and *Ceratitis capitata* (Wiedeman, 1824) (Tephritidae), cause damage to fruit crops in Brazil. Tephritids directly damage the fruit, because the orifice made to lay the eggs causes the fruit to rot and fall prematurely, and the larvae feeding destroy the fruit pulp [1]. Ants, a group of efficient insect predators that regulate populations of general insects [2–8], can be considered as agents of biological pest control in agroecosystems [9–11]. The predation by ants on fruit flies occurs when the larvae leave the fruit in order to bury themselves in the soil and transform into pupae. *Solenopsis geminata* (Fabricius, 1804) ants, for example, were responsible for predation of 95% of the *Anastrepha ludens* (Loew, 1873) larvae during the warm months in Mexico [7]. In Guatemala, these ants attacked 21.6% of the *C. capitata* larvae in orange groves and 9.3% in coffee plantations [8].

Predation is strongly and indirectly influenced by the physical properties of the soil, because the larvae took longer in burying themselves in very dry soil, increasing the time in which they remained exposed and consequently the rate of ant predation [12]. In this study, we analyzed which factors were present and how they influenced the predation of fruit flies by ants, considering the different species of fruit trees, and the degree of soil compaction and moisture content.

2. Material and Methods

We conducted the experiment in a grove of the Universidade Federal da Grande Dourados (UFGD) (Mato Grosso do Sul state, Brazil, 22°13′16″S and 54°48′20″W), on the 8th, 10th, 11th, 14th, 18th and 21st of February 2007. The local

Table 1: Number and total percentage of larvae removed by ants beneath the crowns of 60 trees of three species of fruit, in a grove of the Universidade Federal da Grande Dourados, during an experiment offering groups of three larvae under each crown. Guava is *Psidium guajava*, jaboticaba *Myrciaria jaboticaba*, and mango *Mangifera indica*.

Subfamilies	Species or morphospecies	Fruit trees			Total (%)
		Guava (30 trees and 90 larvae)	Jaboticaba (11 and 33)	Mango (19 and 57)	
Myrmicinae	*Pheidole oxyops* Forel, 1908	7	6	16	67.44
	Pheidole gertrude Forel, 1886	4	—	—	9.30
	Pheidole sp. 1	2	2	—	9.30
	Pheidole sp. 2	1	1	—	4.65
	Pheidole sp. 3	1	—	—	2.32
Dolichoderinae	*Dorymyrmex* sp. 1	—	—	1	2.32
Ponerinae	*Odontomachus chelifer* (Latreille, 1802)	1	—	—	2.32
Ectatomminae	*Ectatomma brunneum* Smith F., 1858	—	—	1	2.32
Total		16	9	18	100

soil is red latosol eutrophic alic [13], and the climate is subtropical humid [14]. In the grove of 4 ha, there are various fruit trees, such as *Psidium guajava* Linn. (Myrtaceae) (popular name guava), *Myrciaria jaboticaba* (Vell.) Berg. (Myrtaceae) (popular name jaboticaba), *Mangifera indica* Linn. (Anacardiaceae) (popular name mango), which we used in this experiment, as well as all fruit trees of grove are arranged in blocks according to species, and only the guava trees had fruit at the time of the experiment. Sixty fruit trees were randomly chosen for the experiment: 30 guavas, 11 jaboticabas, and 19 mangoes. This number is referent to 50% of total individuals of these species of grove.

Beneath the trees' canopies, we delimited an area of 1 m^2 (quadrant) and we removed all vegetal biomass one day before the experimentation to facilitate observation and capture of ants. In each quadrant, we offered simultaneously three last instar larvae *Anastrepha* ssp., obtained from infested guava fruits in the same area of study. We released larvae individually from a height of ~30 cm above the ground, simulating the larva falling from a fruit. During 10 min, from the moment at which the larvae reached the ground, we recorded the time in which the larvae buried themselves, if the larva was attacked and removed by ants (larvae removed), and the time taken by ants to remove them (removal time). All experiments were done at the same period of the day (between 7:00 and 11:00 am), corresponding to the period of the highest incidence of larvae leaving the fruit. Our sampling unit consisted in each larva offered.

After the observations, we collected all ants active in removal of larvae and identified the species according to the dichotomous key of Bolton [15]. Then we stored the ant species in the Laboratório de Mirmecologia of UFGD.

To determine the degree of soil compaction, we used the measure of soil density. We collected 60 soil samples under the canopy of tree after each day of observation. The samples were oven-dried at 110°C for three days. Samples were collected using a metallic cylinder of 4.2 cm in diameter and 5 cm in height. We obtained soil density dividing the dry weight of soil (after 3 days) by the volume of the sample. To determine the soil moisture, we weighed the samples before and after three days in the dryer. The ratio between the initial and final weight multiplied by 100 corresponds to the percentage of moisture. During the days of field study, we recorded the weather conditions, such as daily temperature, relative moisture, and wind speed.

We performed the analysis of covariance (ANCOVA) to verify whether the removal time was dependent on the species of trees, the number of larvae removed, and the interaction between these variables. We used a multivariate analysis of variance (MANOVA) to test the difference in soil compaction and moisture in tree species. We also used models of multiple regression to evaluate if the average time to bury and rate of predation were related to soil moisture, soil compaction, or the interaction between these two variables.

The interaction between the soil characteristics and the species of fruit trees was considered as independent variables. We used multiple regression test to verify whether the time of larvae spent in burying themselves was related to soil moisture and compaction. For this test, we used 30 samples in which the larvae were not predated by ants.

3. Results

From 180 fly larvae used in the experiment, 43 (24%) were removed by ants, 88 (49%) buried themselves, and 49 (27%) did not bury themselves and were not removed by ants. Eight ant species in four genuses and four subfamilies were recorded removing larvae. *Pheidole* (Myrmicinae) accounted for 93% of predation upon larvae (40 records of removal), and individuals of *Pheidole oxyops* Forel, 1908 were the most efficient, removing 67.44% of the larvae (Table 1).

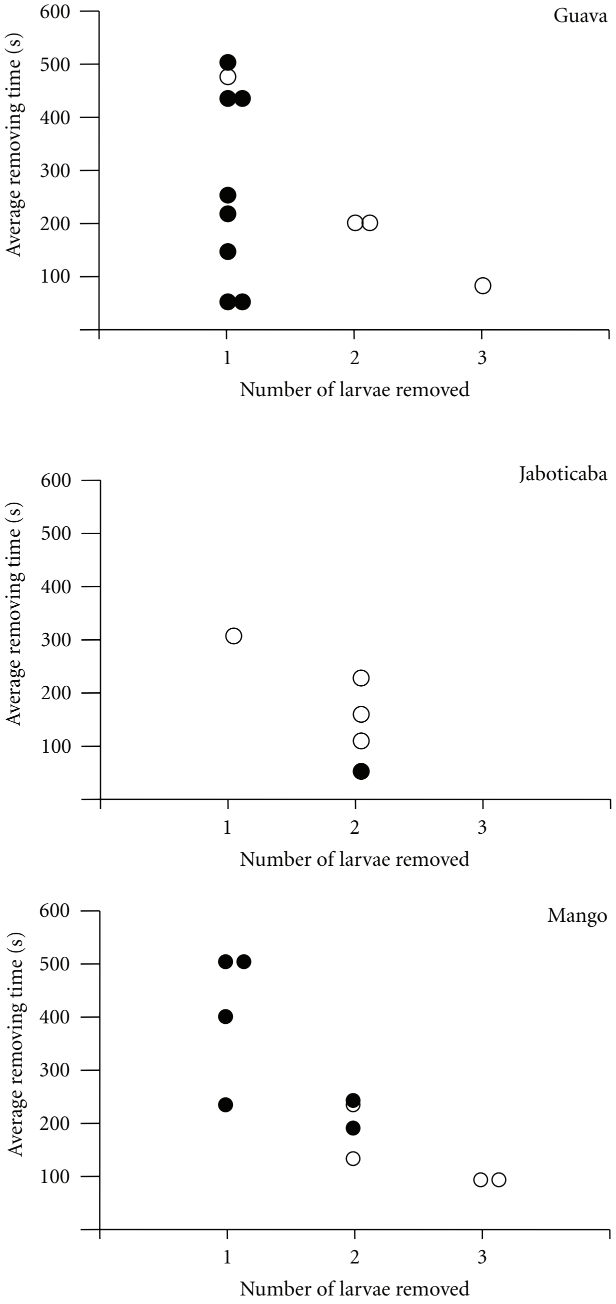

Figure 1: Relationship between amounts of fruit fly larvae (Tephritidae) removed by ants and average time for removal of each larva beneath the crowns of three species of fruit trees. Empty points are samples without larvae burying themselves. Guava is *Psidium guajava*, jaboticaba *Myrciaria jaboticaba*, and mango *Mangifera indica*.

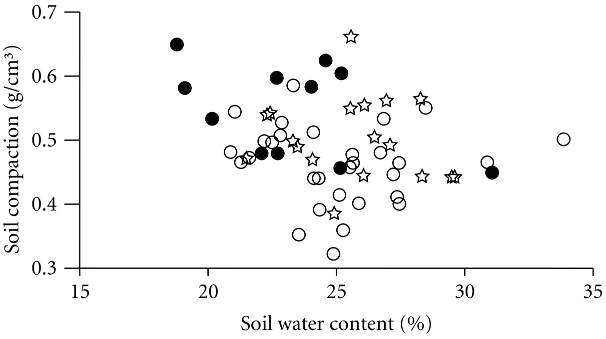

Figure 2: Soil moisture and compaction beneath of crowns the fruit trees. Open circles: guava (*Psidium guajava*); filled circles: jaboticaba (*Myrciaria jaboticaba*); stars: mango (*Mangifera indica*).

The ants removed 16 of these larvae under the canopy of guava trees (all bearing fruit), nine under jaboticabas, and 18 under mangoes. The average time for the larvae to bury themselves was only obtained from 45 samples (26 guavas, six jaboticabas, and 13 mangos), because the larvae in 15 samples did not show this behavior. In 33 samples, there was no attack by ants and the mean of removal time was obtained only from 27 samples (12 guavas, five jaboticabas, and 10 mangos).

The mean time required to remove a larva decreases as the number of larvae attacked and removed increased ($F = 7.356$; $P = 0.013$; gl $= 1$; Figure 1). This significant effect is more evident among samples in which the larvae did not bury themselves (open circles in Figure 1). Moreover, ANCOVA results showed that the removal time was independent of the tree species ($F = 0.894$; $P = 0.424$; gl $= 2$) and the interaction between number of larvae removed and tree species ($F = 0.449$; $P = 0.644$; gl $= 2$). The presence of fruit only in guava did not affect the removal time of the larvae.

Climatic data showed that weather conditions were constant throughout the study. The average daily temperature ranged between 23.7 and 25.5°C, relative moisture varied between 72.4 and 89.6%, and wind speed between 0.8 and 1.6 ms-1. It rained only on the nights of 7th (29.5 mm), 12th (0.3 mm), and 16th (14.2 mm).

The predation rate of larvae was affected by the different soil characteristics, as the larvae take longer to bury themselves in dry soil. Soil compaction and moisture were dependent on tree species (MANOVA: Pillai trace value = 1.195, $P < 0.001$, df = 6 and 112, Figure 2), being that the soil under the jaboticaba canopy had the highest compaction and lower moisture. The average time for burying itself under the different species of fruit trees was significantly related to the soil moisture ($F = 3.803$; $P = 0.037$; gl $= 2$; Figure 3), but not related to soil compaction ($F = 1.052$; $P = 0.366$; gl $= 2$), nor to the interaction between these two variables ($F = 0.553$; $P = 0.582$; gl $= 2$).

Soil characteristics affected the rate of larvae predation by ants (Figure 4). In soils with higher moisture, the predation was lower ($F = 4.753$, $P = 0.021$, df $= 2$), and in more compacted soil, the rate of predation was greater ($F = 5.989$, $P = 0.010$, df $= 2$). Interaction between these two independent variables also explained the predation rate ($F = 6.163$, $P = 0.009$, df $= 2$). In other words, ants were more efficient in preying on larvae on drier and more compact soil, despite compaction having no effect on the larvae burying time (Figure 5).

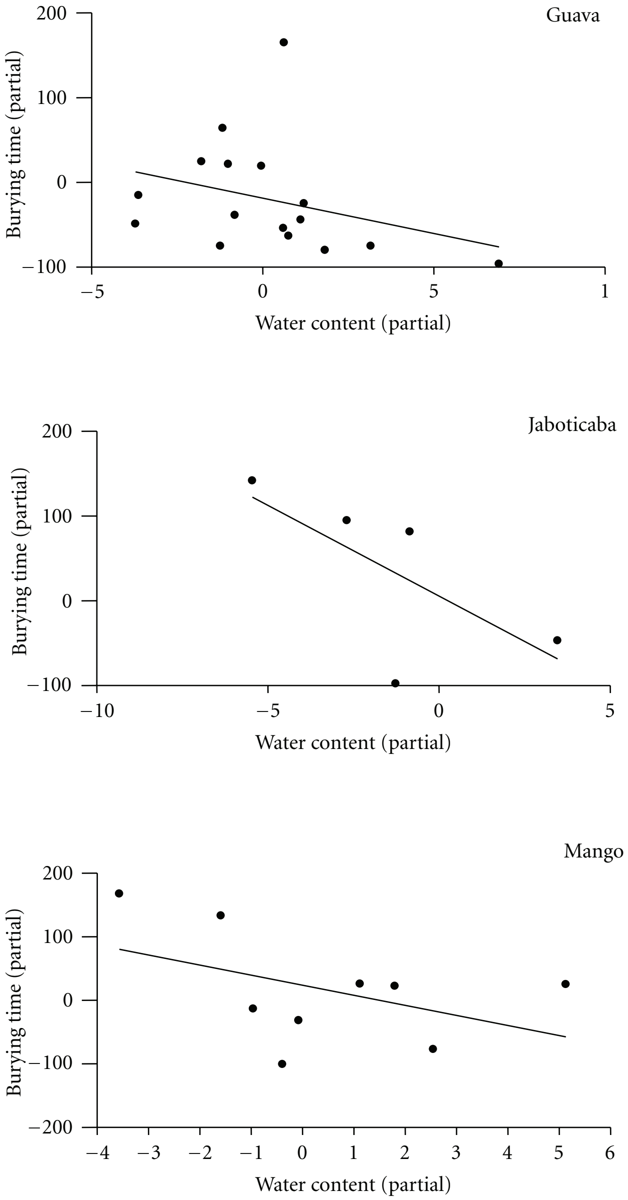

Figure 3: Average time until fruit fly larvae bury themselves through gradient of water content of soil beneath the crowns of three species of fruit trees. Only larvae that were not predated by ants were included. Partial residuals were obtained from a multiple-linear model that included compaction of soil (no significant effect). Guava is *Psidium guajava*, jaboticaba *Myrciaria jaboticaba*, and mango *Mangifera indica*.

4. Discussion

We observed that ants removed approximately 1/4 of the fruit fly larvae released on soil. This value is similar for biological control levels [3, 16, 17] and high for predation of the fruit flies by ants in most studies [8, 12]. Among the predatory ants genus, *Pheidole* individuals were more efficient, accounting for 93% of the larvae removed. The predominance of attacks by these ants evidenced their role as efficient predator, which is also due to their wide distribution, high species richness, and good adaptation to the physical conditions of the environment [18]. Its aggressive behavior and efficient and massive recruitment increment this efficiency [19]. The potential performance of *Pheidole* as agents of biological pest control was also demonstrated in the fight against *Anthonomus grandis* Boheman, 1843 (Coleoptera: Curculionidae) in cotton fields in Brazil [10].

Strategies for predation and defense of organisms are among the most discussed topics in ecology and evolution [20, 21]. These relationships determine the survival or extinction of populations and the structure and maintenance of communities. Thus, if in the case of fruit flies, the rapid penetration into the soil is the best strategy to prevent their predation [7, 12], then the soil characteristics as well as the

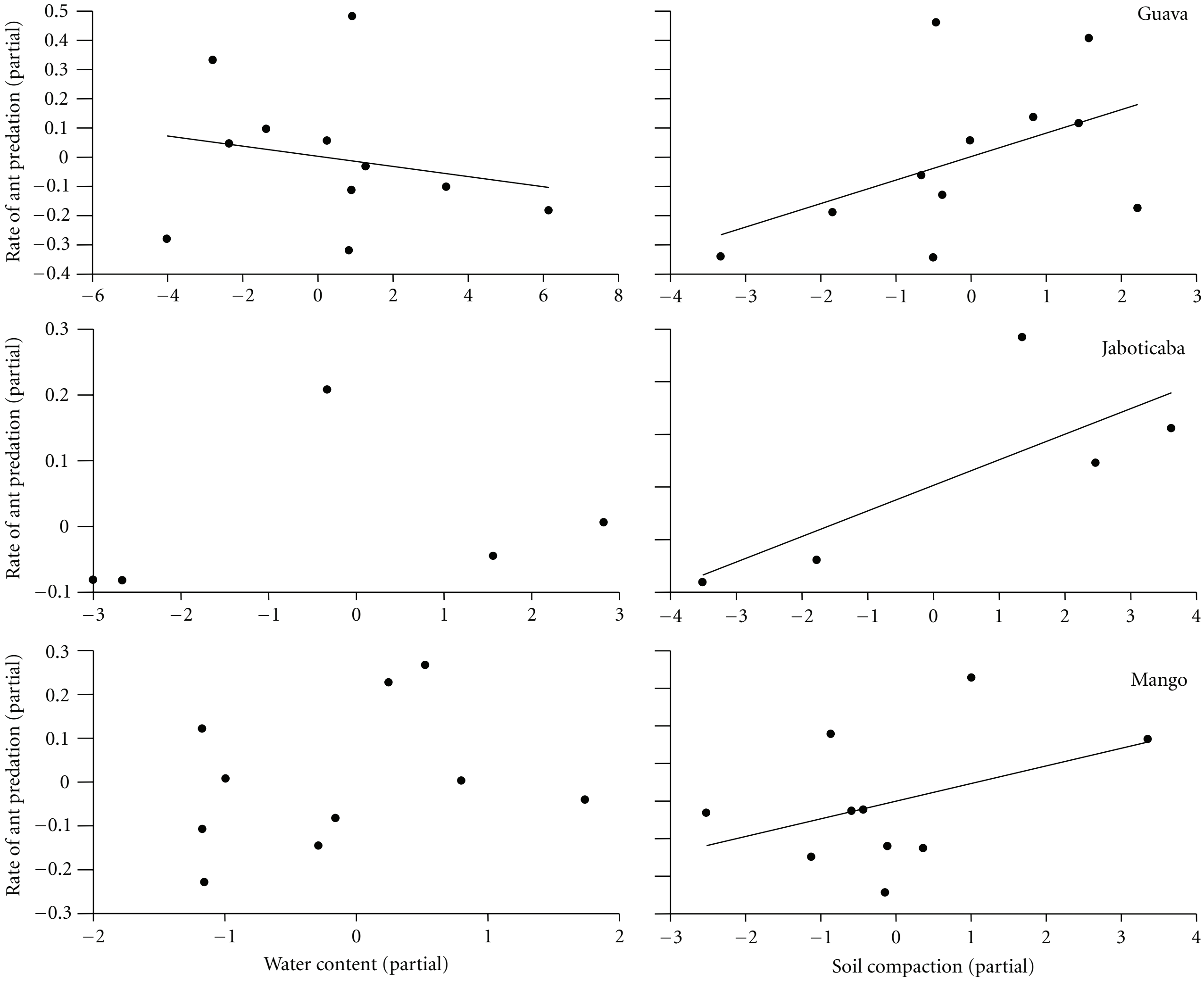

Figure 4: Ant predation on fruit fly larvae through gradients of water content and compaction of soil beneath the crowns of fruit trees of three species. Partial residuals obtained from multiple-linear model. Guava is *Psidium guajava*, jaboticaba *Myrciaria jaboticaba*, and mango *Mangifera indica*.

larvae ability of bury themselves are determinants for their survival. However, in this study, we found that larvae which were dropped on compacted and uncompacted soil took the same time to penetrate the soil. Although the time to drill the soil by larvae was not directly related to compaction, it was significantly associated with soil moisture, another determinant factor for the success of the burying behavior [22, 23] and for the development of pupae [24]. The lack of moisture in the soil can cause mortality of a large number of larvae, because the soils become more difficult to be bored [22]. Wet soils have greater tension between the particles resulting in larger particles and larger spaces among them [25]. Thus, wet soils are more easily bored by fruit fly larvae, as evidenced in this study.

Here we have evidence that both soil tilling and tree species influence the efficiency of ants in attacking the larvae. Several studies have shown that the abundance, not only of ants, but of other predators such as carabid beetles and spiders, increases with farming practices that reduce soil turnover [26, 27]. This fact should be related to environment complexity and colony stability. Tillage systems in which the soil is not turned have a higher plant biomass on the soil surface [28], and this increases the availability of nutrients and shelter for many organisms. Thus, these communities have more local biodiversity [29, 30]. In addition, soil disturbance could have caused the death of various ant colonies, decreasing the number of individuals foraging for resources. Moreover, the tree species may also have influenced the soil characteristics through their complex canopy structure and root density.

Here we showed that rate of ant predation on fruit fly larvae was affected by soil, because larvae took longer to bury themselves in dry and compacted soil. Therefore, the moisture and compaction level of soil, resulting from the type of tillage and tree species, has a profound influence on the burying of larvae influencing the efficiency of ant predation (Figure 5). Nevertheless, the presence of fruit was not a determinant factor in the predation of larvae among the

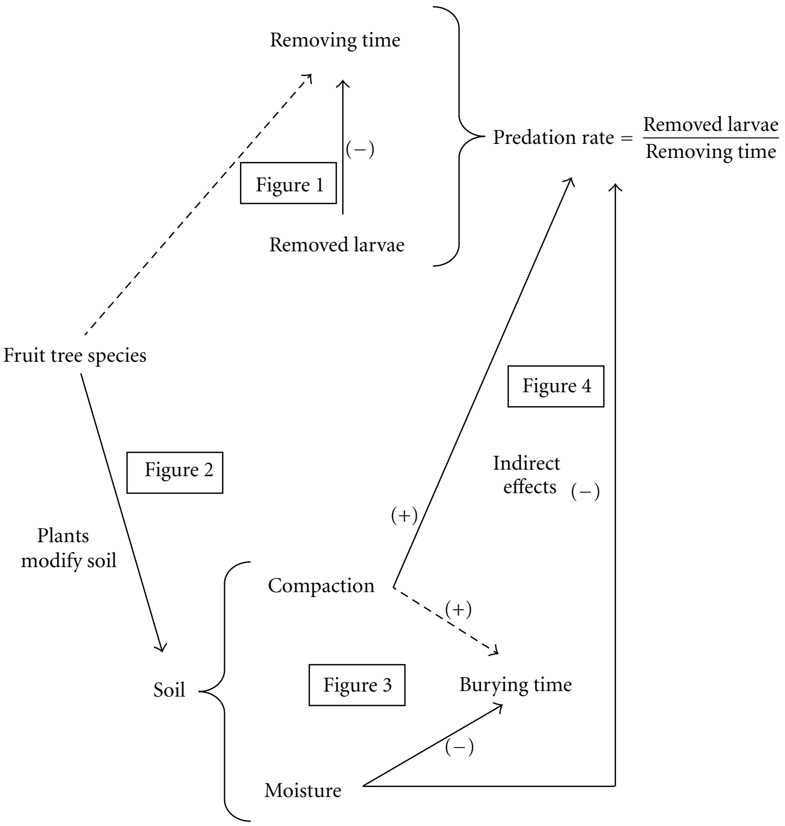

Figure 5: Effects' diagram for predation of fruit fly larvae by ants. Evidences for effects are in the indicated figures. Dashed lines: no statistical evidence; (+): positive effect; (−): negative effect.

fruit trees. This result was also evidenced by Aluja et al. [12]. Although we would expect that ants were more abundant in locations with higher density of fruit, for example, [31], due to the greater number of larvae, only guava trees were bearing fruits at the time of study, which could have masked the effect of fruit.

In this study, we showed that ants, mainly of *Pheidole* genus, are important predators of *Anastrepha* larvae, and can contribute to regulate this crop pest population. Furthermore, we also evidenced that the rate of ant predation depends on soil characteristics and fruit tree species. Thus, ants may have a beneficial impact on fruit growing and, together with other control methods, can reduce cost with insecticides and act as an important tool in integrated pest management.

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Research Article

Floral Resources Used by Euglossini Bees (Hymenoptera: Apidae) in Coastal Ecosystems of the Atlantic Forest

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In spite of playing an important ecological role as pollinators of tropical ecosystems, orchid bees are still poorly known regarding their floral resources. Aiming at a better comprehension of the importance of different plants visited by the Euglossini and, consequently, their role in the maintenance and reproduction of plant species in tropical ecosystems, this study aimed at identifying the flowers visited by those bees in two different areas of the Atlantic Forest in the northern coast of the state of São Paulo, Brazil. Sampling was carried out from August 2007 to July 2009 in two coastal ecosystems in Ubatuba, Brazil. In order to obtain information on flower resources collected by Euglossini bees *in loco*, all bees observed on flowers were collected, pollinaria of Orchidaceae occasionally attached to the body of males were identified, and the pollinic analysis of 68 females was carried out. One hundred twelve bees from 14 species were associated to 105 plant species which represented pollen, nectar, resin, and fragrances sources. These data reinforce the relevance of orchid bees to the maintenance and reproductive success of many tropical plants.

1. Introduction

There is evidence that Euglossini bees play an important ecological role in the maintenance and reproductive success of a wide range of plant species in tropical ecosystems [1–3]. Females visit the plants to collect resin, which is used for nest building as well as nectar and pollen, which are used for provisioning brood cells [4–10]. They have specific foraging routes, known as "traplines," which are followed for several days such that the same flowering plant specimens are visited in a particular sequence. This behaviour implies fidelity to collection sites, and Janzen [11] reported that the females can fly considerable distances quickly, which ensures that a given foraging route can cover a large area. This observation by Janzen [11] is related to the fact that the plants producing food are often widely dispersed in a given area and produce few flowers per day, offering high-quality resources over long periods. Similar to females, Euglossini males are also able to fly quickly and over long distances in search of resources to meet their needs [11–13] and may feed on nectar from the same plants utilised by females [4].

It is estimated that approximately 10% of the 600 to 700 species of the Orchidaceae family are pollinated exclusively by male orchid bees, who visit them to collect floral fragrances [14–17]. These aromatic substances are also collected from plants of several other families, such as Gesneriaceae [18]; Araceae [19]; Euphorbiaceae [20]; Haemodoraceae [21]; Apocynaceae, Loganiaceae, and Malvaceae [22]; Solanaceae [23]; Pentaphylacaceae [24]; Lecythidaceae [25]; Clusiaceae [26]; Amaryllidaceae [27], Bromeliaceae [28], Annonaceae [29], and Plantaginaceae [30], and are stored by males in their modified hind tibias [31, 32] to be released later, presumably to attract females for mating [33, 34].

Plant species from several families have Euglossini as their primary pollinators due to the morphological, ecological, and behavioural characteristics of these bees, including their long tongue, the use of resin for nest construction, the female behaviour of buzz-pollination, and the collection of floral fragrances by males [35–40].

Information on the flora visited by the Euglossini is based on direct observations made *in loco* [35, 41] that are dispersed among bee surveys and studies on plant reproductive biology, many of which are gathered in compilations of data such as the study by Ramírez et al. [42]. It is notable that techniques such as pollen analysis that provide information about the floral resources collected by bees through indirect evidence have been little explored in studies involving Euglossini species [43–45]. Knowledge of the floral resources utilised by the orchid bees enables a better understanding of the importance of the different plants visited during the life cycle of these bees and, consequently, their role in the reproductive biology and maintenance of plant species in tropical ecosystems.

Interaction networks between plants and insects have been used to represent plant-pollinator relationships and to describe the processes, structure, and generalisations of these networks [46, 47]. According to Bezerra et al. [48], the ecological service of pollination is composed of a mosaic of different subservices with a hierarchical structure, of networks within networks, which is particularly evident in phylogenetic groups. Thus, the present study aimed to identify the floral resources collected by Euglossini bees in two Atlantic Forest areas on the northern coast of São Paulo.

2. Materials and Methods

2.1. Study Areas. Samplings were conducted monthly between 9:00 and 15:00 in the period from August 2007 to July 2009 in two areas: the Picinguaba area of Parque Estadual da Serra do Mar (PESM) and Parque Estadual da Ilha Anchieta (PEIA).

The PESM (23°21′51.7′′S, 44°49′56.9′′W) has a total area of 47,500 ha, which contains practically all of the ecosystems that are representative of the Atlantic Forest biome, from mangroves and highly diverse coastal plain vegetation to small areas of high altitude grasslands at its highest elevations, such as the "Pedra do Espelho" (1670 m) and the peaks of Corcovado (1150 m) and Cuscuzeiro (1275 m) in Ubatuba. The Picinguaba area is the only stretch of the PESM that reaches sea level, thus protecting coastal ecosystems.

The PEIA (23°32′25.0′′S, 45°04′15.5′′W) covers a total area of 828 ha, which corresponds to the full extent of Anchieta Island, which is separated by approximately 600 m from the mainland. Many ecosystems of the Atlantic Forest are also present on the island, and the vegetation of these areas was described by Guillaumon et al. [49] and follows Rizzini [50]: anthropic fields (Campo antrópico), whose dominant vegetation is herbaceous and most commonly found families are Poaceae, Melastomataceae, and some ferns; rocky shore, characterised by the predominance of herbaceous and thin vegetation, with high indices of humidity and luminosity, and whose most common families are Araceae, Marantaceae, Sapindaceae, Rubiaceae, Piperaceae, Bromeliaceae, Fabaceae, Cactaceae, and Arecaceae; Atlantic Forest: dense Atlantic Forest in the northeast sector of the island and thin Atlantic Forest in the southwest, which are predominantly composed of the families Fabaceae, Arecaceae, Rubiaceae, Melastomataceae, Meliaceae, Salicaceae, Clusiaceae, Araceae, Malvaceae, Bignoniaceae, Piperaceae, Polypodiaceae, and Urticaceae; *Gleichenia* areas: dense patches of vegetation consisting of individuals of the Gleicheniaceae family; mangroves: vegetation that is not very substantial, with some individuals of *Avicennia* L. (Acanthaceae) and *Acrostichum* L. (Pteridaceae); restinga: a coastal vegetation that varies from sparse to transitional broadleaf forest, and whose most commonly observed families are Bromeliaceae, Myrtaceae, Anacardiaceae, Cyperaceae, Araceae, Cactaceae, Poaceae, Fabaceae, Arecaceae, Polypodiaceae, and Gleicheniaceae [51].

2.2. Samplings of Bees. Three sampling methods were used in order to identify the floral sources visited by orchid bees: sampling of males using scent baits, observations, and collections of both females and males on flowers and the pollen analysis of females collected both on flowers, in flight or at scent baits.

Male bees' samplings by scent baits were performed along two distinct trails: the "Picadão da Barra" Trail (23°21′51.7′′S, 44°49′56.9′′W, altitude 3 m), which was used in the first year of study between August 2007 and July 2008, and the "Guanambi" Trail (23°21′37.0′′S, 44°50′52.9′′W, altitude 3 m), where censuses were conducted in the second year, between August 2008 and July 2009. As in the Picinguaba area, two trails were utilised for the census of males with scent baits: in the first year, the "Praia das Palmas" Trail (23°32′25.0′′S, 45°04′15.5′′W, sea level) and, in the second year of samplings, the "Represa" Trail (23°32′27.3′′S, 45°03′58.9′′W, altitude 18 m).

To obtain information on the floral resources collected by both females (pollen, resin, and nectar) and males (nectar and floral fragrances), all of the flowering species present within 50 m of the trails used for the collection of males with scent baits were observed, monitored, and identified. The collection behaviour of bees as well as the resources collected was also recorded.

2.3. Pollinaria. During the screening of the collected material, Orchidaceae pollinaria that were occasionally found attached to the bodies of the males were removed and placed in individual vials containing 70% alcohol for preservation and identification.

2.4. Pollen Analysis. Due to the scarcity of pollen grains in males, pollen analysis was performed only for the 68 collected females, which were distributed among nine species: *Eufriesea dentilabris* (Mocsáry, 1897) ($N = 2$), *Eufriesea smaragdina* (Perty, 1833) ($N = 1$), *Eufriesea surinamensis* (Linnaeus, 1758) ($N = 1$), *Euglossa cordata* (Linnaeus, 1758) ($N = 8$), *Euglossa stellfeldi* (Moure, 1947) ($N = 5$), *Euglossa townsendi* (Cockerell, 1904) ($N = 1$), *Eulaema cingulata* (Fabricius, 1804) ($N = 4$), *Eulaema helvola* (Moure, 2003) ($N = 4$), and *Eulaema seabrai* (Moure, 1960) ($N = 46$). The contents of the corbiculae of these females were removed with forceps and placed in vials containing absolute glacial acetic acid for subsequent mounting on slides for light microscopy according to the acetolysis protocol described by Erdtman [52]. After the process of acetolysis, pollen grains were placed in test tubes containing 50% glycerine for at least 24 hours. Small amounts of acetolysed material were deposited on slides containing glycerol gelatine.

To identify the types of pollen on the slides, samples were collected from the anthers of herbarium specimens deposited at the Herbarium of the Universidade Estadual de Campinas (UEC), the Herbarium Rioclarense (HRCB), and the Herbarium of the Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (SPFR). The identification of the pollen grains as pollen, resin, or nectar sources was carried out based on the occurrence of the pollen types at the slides and also according to the floral morphology and the available floral resources of the plant species. The morphology of orchid bee species and literature information concerning their collection behaviour on flowers were also considered. Many of these herbarium specimens came from floristic surveys and/or collections conducted in Ubatuba, around Picinguaba and on Anchieta Island, and were accessed through the SpeciesLink network database [53]. The slides made with the anthers removed from herbarium specimens as well as those with anthers collected locally, were deposited at the Laboratório de Palinoecologia of the Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto.

2.5. Interaction Networks. Interaction networks were constructed using qualitative interaction matrices for Euglossini males and females and the plants that these bees used as resources. In the matrices, the rows represented plant species and the columns represented the bee species. The matrices recorded the presence (1) or absence (0) of an interaction between a given plant and a bee species. Collections of resin and fragrance were also considered interactions. Networks representing the interactions established between bees and pollen sources (females only) and nectar (males and females) were also analyzed to better assess the types of interactions established. Resin and fragrance were not plotted because both resources occurred in a few interactions and they were only represented at Figure 1. The significance of NODF was estimated using a Monte Carlo procedure with 1000 randomizations using the null model Ce in the computer software ANINHADO 3.0 [54]. The interaction networks were constructed in the form of bipartite graphs by the bipartite package of the R program [55]. Connectance was also calculated using the same package. Because collections were carried out in the same biome (Atlantic Forest) using the same methodology and sampling effort, the data were analysed together.

3. Results

3.1. Observations in Flowers. In the Atlantic coastal ecosystems studied, 112 bees were collected belonging to 14 species, which were associated with 105 species of plants. Ten Euglossini species, represented by 79 individuals, were sampled in a total of 21 plants in the Ubatuba region (Table 1). In Picinguaba, ten bee species were sampled: *Ef. dentilabris, Ef. surinamensis, Euglossa iopoecila* Dressler, 1982, *Eg. stellfeldi, Eg. townsendi, El. cingulata,* and *El. seabrai*, whereas on Anchieta Island, the species *Ef. smaragdina*, *Eg. cordata*, *Eg. stellfeldi, El. cingulata, El. helvola,* and *El. seabrai* visited the flowers of ten plants. In addition to the two areas studied, there is also a record of *El. seabrai* males on inflorescences of *Anthurium andraeanum* at the base of the Instituto Oceanográfico da Universidade de São Paulo–IO/USP, located near the "Saco da Ribeira" marina, which is the point of access for Anchieta Island.

Regarding the floral resources collected, five species of flowers were visited exclusively by females to collect pollen, and 15 plants were used as sources of nectar for both males and females (Table 1). On Anchieta Island, in addition to the native species of the Atlantic Forest biome, exotic plants were also visited by Euglossini bees to collect floral resources. These exotic species included ornamental *Alpinia zerumbet, Thunbergia alata,* and *Impatiens walleriana* as well as papaya, *Carica papaya*.

3.2. Pollinaria. Of the 1503 males collected in artificial baits [56], only 32 (2.13%), represented by eight species, carried pollinaria of ten Orchidaceae species (Table 2). Ten orchid species from eight genera were identified, and only the pollinaria of *Catasetum hookeri* and *Cirrhaea fuscolutea* were found in males from more than one Euglossini species (Table 2). With the exceptions of *Catasetum hookeri*, *Catasetum socco*, *Cirrhaea dependens,* and *Cirrhaea fuscolutea*, all other orchids were found exclusively in one study area (Table 2). All species of Orchidaceae found in the present study provide only floral fragrances as a resource for male Euglossini visiting their flowers (R. B. Singer, pers. comm.).

3.3. Pollen Analysis. Of the 68 females whose pollen grains were collected for pollen analysis, only four of them (all *Eg. stellfeldi*) carried no pollen grains. With the exception of females collected in scent baits (15 *El. seabrai* females and one *Eg. cordata*) or captured in flight (a female of *El. cingulata* and one of *El. seabrai*), the other 46 samples were from females that visited flowers. Most of the material analysed was collected at Anchieta Island, which included 50 samples from six species (*Ef. smaragdina*, *Eg. cordata*, *Eg. stellfeldi*, *El. cingulata*, *El. helvola,* and *El. seabrai*); only 14 samples of four species (*Ef. dentilabris*, *Ef. surinamensis*, *Eg. townsendi,* and *El. seabrai*) were obtained from Picinguaba.

In total, 87 pollen types were identified belonging to 68 species from 24 families and 19 unidentified taxa. These 19 taxa were found in pollen samples from *Ef. surinamensis* (two taxa), *Eg. cordata* (four taxa), *Eg. stellfeldi* (two taxa), *Eg. townsendi* (one taxon), *El. seabrai* (12 taxa), *El. helvola,* and *El. cingulata* (one taxon each). Four of the unidentified taxa were found in pollen samples from eight bee species. Of the 15 plant species in which females were collected on flowers, only pollen grains of *Alpinia zerumbet*, *Ipomoea tiliacea,* and *Temnadenia odorifera* were not found in the samples, whereas pollen from *Canistropsis seidelii*, a species that was visited by

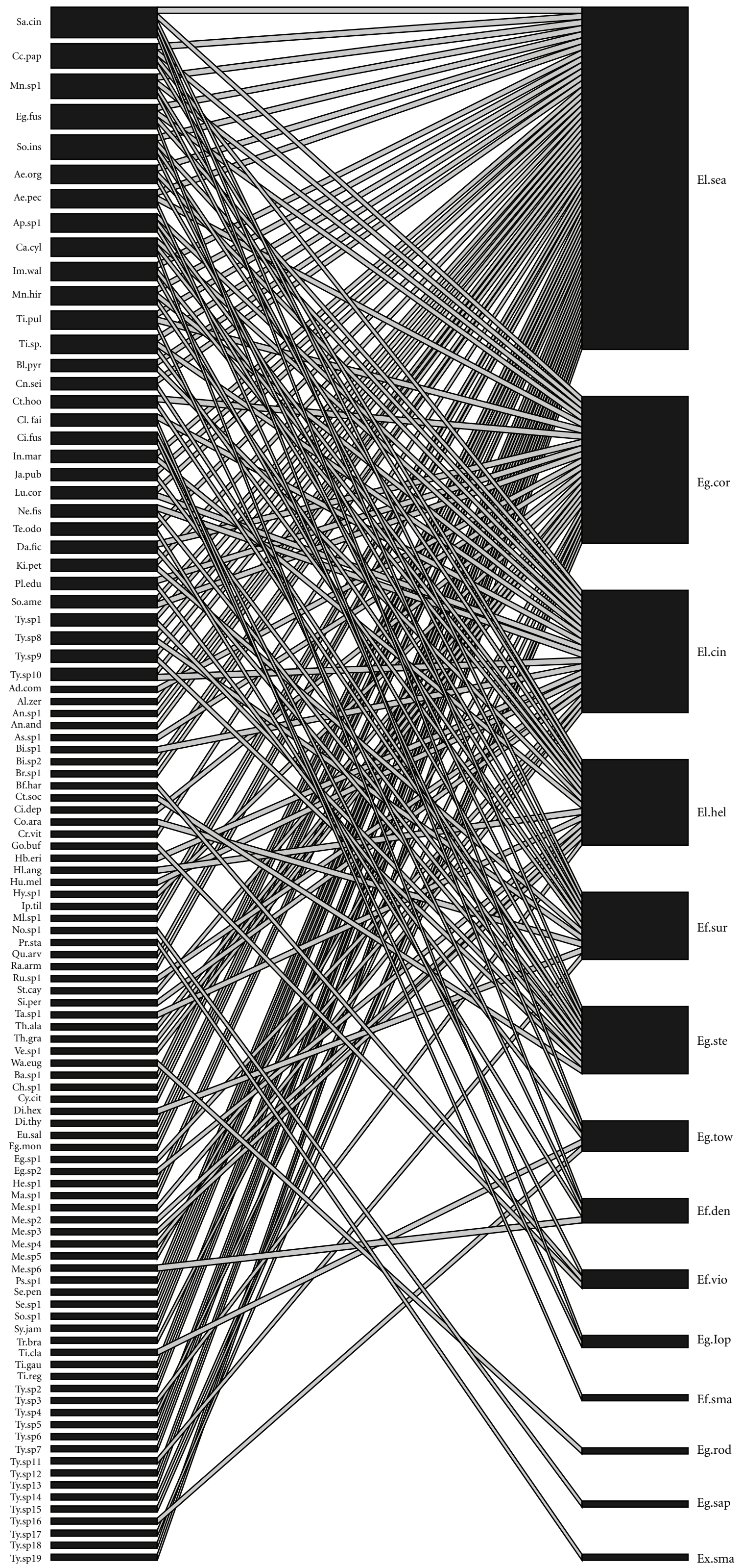

Figure 1: Network of interactions between plants and bees of the Euglossini tribe in two areas of the Atlantic Forest: Picinguaba and Anchieta Island, Ubatuba, SP. Bee species: El.sea—*El. seabrai*; Eg.cor—*Eg. cordata*; El.cin—*El. cingulata*; El.hev—*El. helvola*; Ef.sur—*Ef. surinamensis*; Eg.ste—*Eg. stellfeldi*; Eg.tow—*Eg. townsendi*; Ef.den—*Ef. dentilabris*; Ef.vio—*Ef. violacea*; Eg.iop—*Eg. iopoecila*; Ef.sma—*Ef. smaragdina*; Eg.rod—*Eg. roderici*; Eg.sap—*Eg. sapphirina*; Ex.sma—*Ex. smaragdina*. Plant species abbreviations are given at Table 3.

Table 1: Plant species visited and flower resources collected by Euglossini bees around Ubatuba between August 2007 and July 2009.

Families	Plant species	Resources	Areas
Acanthaceae	*Thunbergia alata* Bojer *ex* Sims	Nectar	Anchieta Island
Apocynaceae	*Mandevilla hirsuta* (A.Rich.) K.Schum.	Nectar	Picinguaba
	Temnadenia odorifera (Vell.) J.F.Morales	Nectar	Picinguaba
Araceae	*Anthurium andraeanum* Linden	Fragrance	IO/USP
Balsaminaceae	*Impatiens walleriana* Hook.f.	Nectar	Anchieta Island
Bignoniaceae	*Adenocalymma comosum* (Cham.) DC.	Nectar	Anchieta Island
	Jacaranda puberula Cham.	Nectar	Picinguaba
Bromeliaceae	*Canistropsis seidelii* (L.B.Smith and Reitz) Leme	Nectar	Picinguaba
Caricaceae	*Carica papaya* L.	Nectar	Anchieta Island
Commelinaceae	*Dichorisandra hexandra* (Aubl.) Kuntze *ex* Hand.-Mazz.	Pollen	Picinguaba
Convolvulaceae	*Ipomoea tiliacea* (Willd.) Choisy	Nectar	Picinguaba
Fabaceae	*Clitoria fairchildiana* R.A.Howard	Nectar	Anchieta Island
Marantaceae	*Calathea cylindrica* (Roscoe) K.Schum.	Nectar	Anchieta Island
	Calathea monophylla (Vell.) Körn.	Nectar	Picinguaba
Melastomataceae	*Tibouchina clavata* (Pers.) Wurdack	Pollen	Picinguaba
	Tibouchina regnellii Cogn.	Pollen	Picinguaba
Pentaphylacaceae	*Ternstroemia brasiliensis* Cambess.	Pollen	Anchieta Island
Rubiaceae	*Sabicea cinerea* Aubl.	Nectar	Picinguaba
Solanaceae	*Solanum insidiosum* Mart.	Pollen	Anchieta Island
Verbenaceae	*Stachytarpheta cayennensis* (Rich.) Vahl	Nectar	Anchieta Island
Zingiberaceae	*Alpinia zerumbet* (Pers.) B.L.Burtt and R.M.Sm.	Nectar	Anchieta Island

Table 2: Species of Orchidaceae whose pollinaria were found attached to the bodies of male Euglossini collected in Picinguaba and Anchieta Island, Ubatuba, SP. P: Picinguaba, A: Anchieta Island.

Orchids species	Euglossine species	Areas	$N = 32$*
Bifrenaria harrisoniae (Hook.) Rchb.f.	*Ef. violacea* (1♂)	A	1*
Catasetum hookeri Lindl.	*Eg. cordata* (2♂), *Eg. stellfeldi* (7♂)	A[1], P[2]	9
Catasetum socco (Vell.) Hoehne	*Eg. stellfeldi* (5♂)	A[3], P[4]	5
Cirrhaea dependens (Lodd.) Loudon	*Eg. cordata* (11♂)	A[5], P[6]	11
Cirrhaea fuscolutea Lindl.	*Ef. violacea* (1♂), *Eg. iopoecila* (2♂)	A[7], P[8]	3*
Gongora bufonia Lindl.	*Ef. violacea* (1♂)	A	1*
Huntleya meleagris Lindl.	*El. cingulata* (1♂)	P	1
Notylia sp.	*Eg. sapphirina* (1♂)	P	1
Promenaea stapelioides (Link. and Otto) Lindl.	*Ex. smaragdina* (1♂)	P	1
Warmingia eugenii Rchb.f.	*Eg. roderici* (1♂)	P	1

* Only one individual of *Ef. violacea* was collected, which carried pollinaria of *Bifrenaria harrisoniae*, *Cirrhaea fuscolutea*, and *Gongora bufonia*.
[1]5 *Eg. stellfeldi* males and 1 *Eg. cordata* male, [2]2 *Eg. stellfeldi* males and 1 *Eg. cordata* male, [3]2 males, [4]3 males, [5]1 male, [6]10 males, [7]1 *Ef. violacea* male and 1 *Eg. stellfeldi* males, [8]2 *Eg. iopoecila* males.

only one male of *Eg. stellfeldi*, was identified from the slides. Among all of the species of Euglossini, *El. seabrai* showed the greatest number of pollen types (51) followed by *Eg. cordata* (21) and *El. cingulata* (16).

Pollen was collected from 33 species represented by eight families: Myrtaceae ($N = 9$ species), Clusiaceae ($N = 1$), Pentaphylacaceae ($N = 1$), Malpighiaceae ($N = 3$), Solanaceae ($N = 3$), Melastomataceae ($N = 11$), Fabaceae (subfamily Caesalpinioideae) ($N = 3$), and Commelinaceae ($N = 2$). *Dalechampia ficifolia* was the only source of resin identified in the samples from *Eg. cordata* and *Eg. stellfeldi*. The 34 species of plants identified as nectar sources were distributed among the families Acanthaceae ($N = 2$), Amaranthaceae ($N = 1$), Apocynaceae ($N = 3$), Asteraceae ($N = 2$), Balsaminaceae ($N = 1$), Bignoniaceae ($N = 5$), Bromeliaceae ($N = 6$), Caricaceae ($N = 1$), Costaceae

Table 3: Abbreviated plant names used to construct the interactions are given. The 19 unidentified taxa were abbreviated as Ty. sp1, Ty. sp2, and so on.

Families/species	Abbreviations
Acanthaceae	
Thunbergia alata Bojer *ex* Sims	Th.ala
Thunbergia grandiflora Roxb.	Th.gra
Amaranthaceae	
Hebanthe eriantha (Poir.) Pedersen	Hb.eri
Apocynaceae	
Mandevilla hirsuta (A.Rich.) K.Schum.	Mn.hir
Mandevilla sp.1	Mn. sp1
Temnadenia odorifera (Vell.) J.F.Morales	Te.odo
Type 1	Ap. sp1
Araceae	
Anthurium andraeanum Linden	An.and
Asteraceae	
Type 1	As. sp1
Vernonanthura sp.1	Ve. sp1
Balsaminaceae	
Impatiens walleriana Hook.f.	Im.wal
Bignoniaceae	
Adenocalymma comosum (Cham.) DC.	Ad.com
Jacaranda puberula Cham.	Ja.pub
Lundia cordata (Vell.) DC.	Lu.cor
Stizophyllum perforatum (Cham.) Miers	Si.per
Tabebuia sp.1	Ta. sp1
Type 1	Bi. sp1
Type 2	Bi. sp2
Bromeliaceae	
Aechmea organensis Wawra	Ae.org
Aechmea pectinata Baker	Ae.pec
Billbergia pyramidalis (Sims) Lindl.	Bl.pyr
Canistropsis seidelii (L.B.Sm. and Reitz) Leme	Cn.sei
Quesnelia arvensis (Vell.) Mez	Qu.arv
Type 1	Br.sp1
Caricaceae	
Carica papaya L.	Cc.pap
Clusiaceae	
Kielmeyera petiolaris Mart.	Ki.pet
Commelinaceae	
Dichorisandra hexandra (Aubl.) Kuntze *ex* Hand.-Mazz.	Di.hex
Dichorisandra thyrsiflora J.C.Mikan	Di.thy
Convolvulaceae	
Ipomoea tiliacea (Willd.) Choisy	Ip.til
Costaceae	
Costus arabicus L.	Co.ara
Euphorbiaceae	
Dalechampia ficifolia Lam.	Da.fic
Fabaceae	
Andira sp.1	An. sp1
Clitoria fairchildiana R.A.Howard	Cl.fai
Crotalaria vitellina Ker Gawl.	Cr.vit
Inga marginata Willd.	In.mar
Senna pendula (Humb. and Bonpl. *ex* Willd.) H.S.Irwin and Barneby	Se.pen
Senna sp.1	Se. sp1
Type *Chamaecrista*	Ch. sp1
Gesneriaceae	
Nematanthus fissus (Vell.) L.E.Skog	Ne.fis
Heliconiaceae	
Heliconia angusta Vell.	Hl.ang
Lamiaceae	
Hyptis sp.1	Hy. sp1
Malpighiaceae	
Heteropterys sp.1	He. sp1
Type *Banisteriopsis*	Ba. sp1
Type 1	Ma. sp1
Malvaceae	
Type 1	Ml. sp1
Marantaceae	
Calathea cylindrica (Roscoe) K.Schum.	Ca.cyl
Calathea monophylla (Vell.) Körn.	Ca.mon
Melastomataceae	
Tibouchina clavata (Pers.) Wurdack	Ti.cla
Tibouchina gaudichaudiana (DC.) Baill.	Ti.gau
Tibouchina cf. *pulchra* Cogn.	Ti.pul
Tibouchina regnellii Cogn.	Ti.reg
Tibouchina sp.1	Ti. sp1
Type 1	Me. sp1
Type 2	Me. sp2
Type 3	Me. sp3
Type 4	Me. sp4
Type 5	Me. sp5
Type 6	Me. sp6
Myrtaceae	
Corymbia citriodora (Hook.) K.D.Hill and L.A.S.Johnson	Cy.cit
Eucalyptus saligna Sm.	Eu.sal
Eugenia cf. *fusca* O.Berg	Eg.fus
Eugenia cf. *monosperma* Vell.	Eg.mon
Eugenia sp.1	Eg. sp1
Eugenia sp.2	Eg. sp2
Plinia edulis (Vell.) Sobral	Pl.edu
Psidium sp.1	Ps. sp1
Syzygium jambos (L.) Alston	Sy.jam
Orchidaceae	
Huntleya meleagris Lindl.	Hu.mel
Notylia sp.	No. sp1
Promenaea stapelioides (Link. and Otto) Lindl.	Pr.sta
Warmingia eugenii Rchb.f.	Wa.eug

Table 3: Continued.

Families/species	Abbreviations
Pentaphylacaceae	
Ternstroemia brasiliensis Cambess.	Tr.bra
Rubiaceae	
Randia armata (Sw.) DC.	Ra.arm
Sabicea cinerea Aubl.	Sa.cin
Type 1	Ru. sp1
Solanaceae	
Solanum americanum Mill.	So.ame
Solanum insidiosum Mart.	So.ins
Solanum sp.1	So. sp1
Verbenaceae	
Stachytarpheta cayennensis (Rich.) Vahl	St.cay
Zingiberaceae	
Alpinia zerumbet (Pers.) B.L.Burtt and R.M.Sm.	Al.zer

($N = 1$), Fabaceae (subfamilies Mimosoideae ($N = 1$) and Faboideae ($N = 3$)), Gesneriaceae ($N = 1$), Heliconiaceae ($N = 1$), Lamiaceae ($N = 1$), Malvaceae ($N = 1$), Rubiaceae ($N = 3$), and Verbenaceae ($N = 1$).

Samples of pollen removed from females from Anchieta Island revealed the presence of exotic species such as *Thunbergia alata*, *Thunbergia grandiflora*, *Corymbia citriodora*, and *Eucalyptus saligna*, as well as *Impatiens walleriana* and *Carica papaya*, which were exploited as sources of nectar.

3.4. Interaction Networks. A total of 153 qualitative interactions between 105 species of plants and 14 Euglossini species were identified in the present study. The interaction network was nested (NODF 15.46, $P > 0.05$), the network connectance was relatively high (0.106), with the asymmetry of the interaction network evident in the bipartite graphical representation (Figure 1), where a few species had many interactions and many species were attributed with a single interaction. Generally, plants had few interactions, with *Sabicea cinerea* connected to five Euglossini species and *Carica papaya*, *Mandevilla* sp., *Eugenia* cf. *fusca*, and *Solanum insidiosum* to four species. Seventy-two plant species showed only one network interaction. Bees, on the other hand, showed a much higher number of interactions, with *El. seabrai* being connected to 55 plant species, representing 35% of interactions. *Euglossa cordata* and *El. cingulata* also stood out in their numbers of interactions with 24 and 20 interactions, respectively. *Eufriesea smaragdina*, *Eg. roderici*, *Eg. sapphirina,* and *Ex. smaragdina* showed only a single network interaction each. All of the types of resources offered by plants to male and female Euglossini are included in the interactions described.

Most of the observed interactions were based on two types of resources, pollen and nectar, which were responsible for 46% and 45% of interactions, respectively. Fragrance resources were responsible for 8% of the interactions, and resin contributed to only 1% of the total interactions. Only three instances were recorded of males and females sharing the same plant species in search of the same type of resource, nectar. These instances were *Eg. stellfeldi* x *Impatiens walleriana*, *El. seabrai* x *Impatiens walleriana,* and *El. cingulata* x *Clitoria fairchildiana*.

The interaction networks grouped by nectar (Figure 2) was also nested (NODF 23.24, $P > 0.05$), whereas the pollen network (Figure 3) was not significantly nested (NODF 22.36, $P = 0.38$). Analyzing the interactions of bees with the type of resource, nectar and pollen, offered by the plants, it is possible to observe alternation in plant species that offer each type of resource, and in both cases there is a marked predominance of one plant species. Regarding bees it is possible to observe in both types of floral resources the dominance of some bee species by the offered resource. The species *El. seabrai* interacted with the largest number of nectar resources (22), followed by *El. cingulata* (12) and *Eg. cordata* (11) (Figure 2). The bee species that had the highest number of interactions with plants that provide pollen as floral rewards were *Ef. dentilabris* (21), *Ef. surinamensis* (6) and, *Eg. cordata* (6).

4. Discussion

As reported for other Euglossini species [35, 39, 43, 44, 57, 58], the species sampled in Ubatuba can also be characterised as polylectic. This conclusion is supported not only by the records of male and female bees on flowers but also the analysis of pollen loads from the corbiculae and other parts of the bodies of females, from which more than 100 plant species exploited as sources of pollen, nectar, resin, and floral fragrances were identified.

Results from the present study support findings from previous studies that report that species of plants from the Solanaceae, Fabaceae, and Melastomaceae families are among the most important sources of pollen for Euglossini species [44, 57–60]. This information strongly suggests that Euglossini females exhibit certain consistency in collecting pollen, particularly from species whose flowers have poricidal anthers. According to Roulston et al. [61], the protein content of pollen from these plants can reach up to 48%, a substantial value that lies within the range of the percentage of protein, 12–61%, found in grains of pollen usually collected by bees.

In addition to species of the families Solanaceae, Fabaceae, and Melastomaceae, Myrtaceae species are also visited to obtain pollen, as observed in the present study and reported by Bittrich et al. [62] for *Euglossa* sp., by Torezan-Silingardi and Del-Claro [63] for *El. nigrita*, and Falcão et al. [64] for *Eulaema mocsaryi* (Friese, 1899). Euglossini bees pollinate species lacking anthers with poricidal dehiscence through a process of vibrating the anthers, known as "buzz-pollination." The Euglossini are also important pollinators of species of *Kielmeyera* Mart. & Zucc. (Guttiferae) [65, 66], which, like the Myrtaceae, also have flowers with numerous stamens and longitudinal anthers. "Buzz-pollination" behaviour allows for a smaller number of visits by bees to these flowers in addition to being a more efficient method

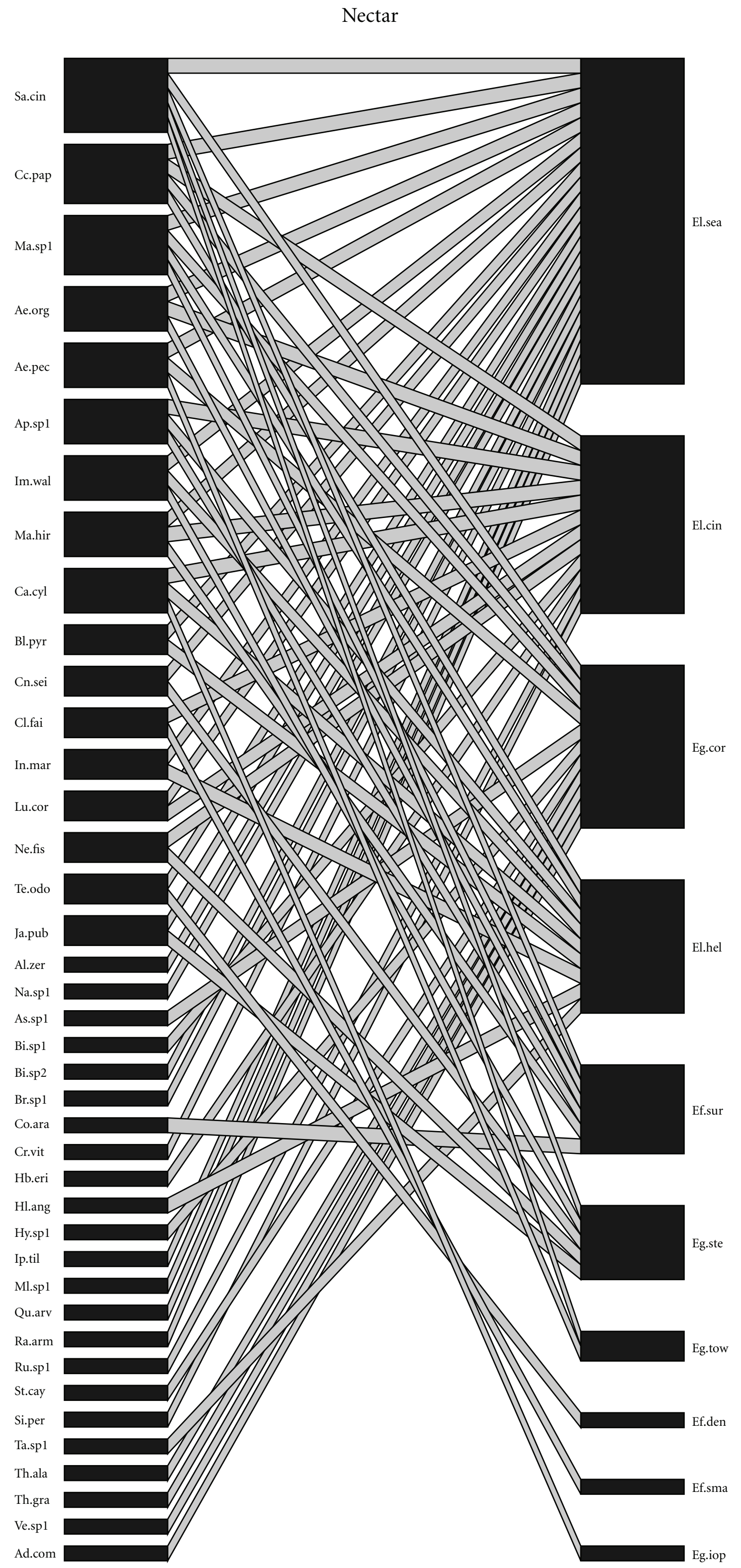

Figure 2: Interaction networks between plants and bees of the Euglossini tribe, grouped by the nectar resources that were exploited both by females and males in two areas of Atlantic Forest, Picinguaba and Anchieta Island, Ubatuba, SP. Bee species: El.sea—*El. seabrai*; El.cin—*El. cingulata*; Eg.cor—*Eg. cordata*; El.hev—*El. helvola*; Ef.sur—*Ef. surinamensis*; Eg.ste—*Eg. stellfeldi*; Eg.tow—*Eg. townsendi*; Ef.den—*Ef. dentilabris*; Ef.sma—*Ef. smaragdina*; Eg.iop—*Eg. iopoecila*. Plant species abbreviations are given at Table 3.

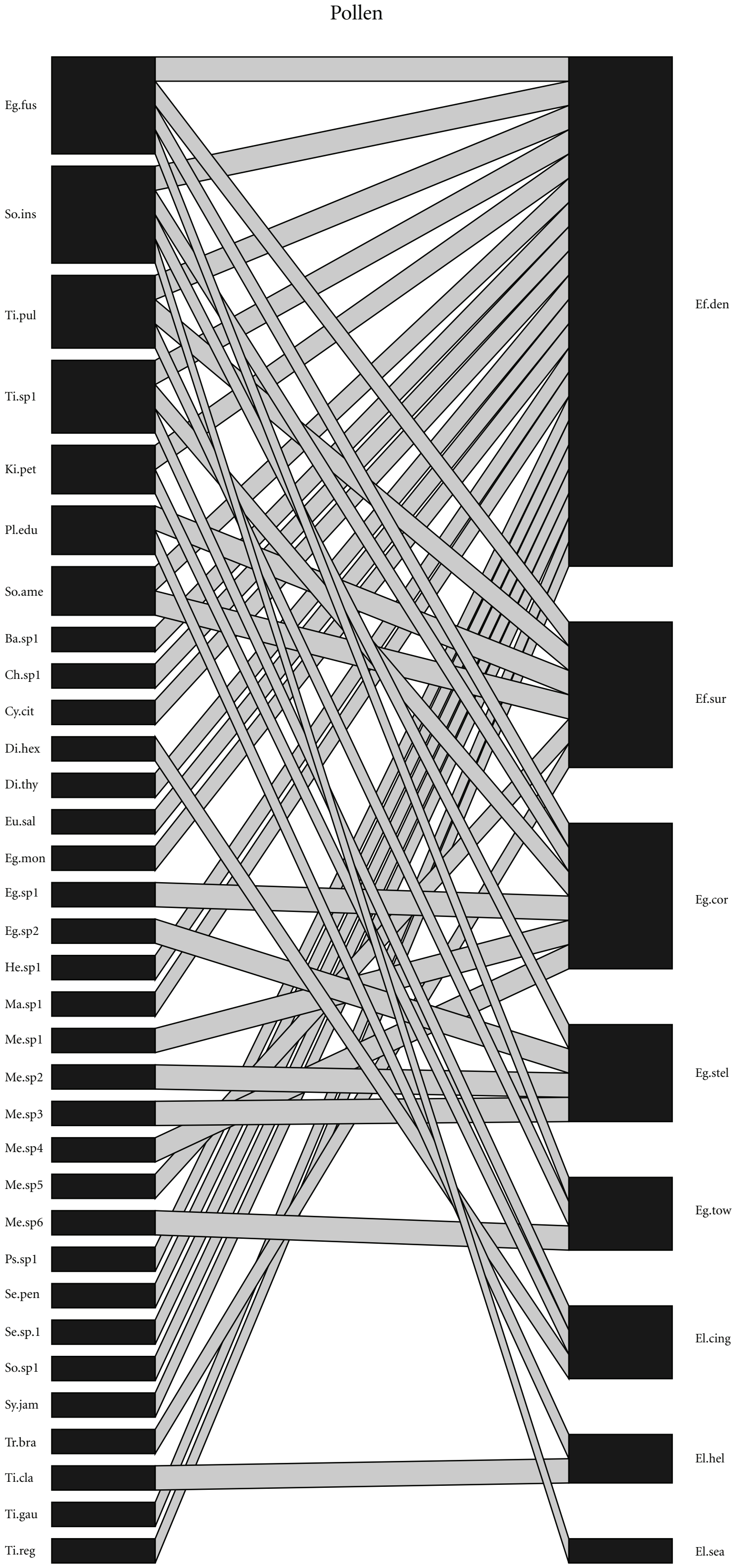

Figure 3: Interaction networks between plants and bees from the Euglossini tribe, grouped by the pollen resources that were exploited solely by females in two areas of Atlantic Forest: Picinguaba and Anchieta Island, Ubatuba, SP. Bee species: Ef.den—*Ef. dentilabris*; Ef.sur—*Ef. surinamensis*; Eg.cor—*Eg. cordata*; Eg.ste—*Eg. stellfeldi*; Eg.tow—*Eg. townsendi*; El.cin—*El. cingulata*; El.hev—*El. helvola*; El.sea—*El. seabrai*. Plant species abbreviations are given at Table 3.

to obtain pollen, as a greater number of stamens are joined and more anthers are vibrated at once [65]. According to Buchmann [36], there are rare cases where the pollen is collected through sonication in flowers with anthers lacking poricidal openings; there are, as yet, no definite causes for this, which could also be a "side effect" of the behaviour of the bees.

Although the stems of some species, such as *Protium* Burm.f. (Burseraceae), *Anacardium* L., and *Spondias* L. (Anacardiaceae), can be scraped by Euglossini females to obtain resin [4, 57], species of *Clusia* L. (Clusiaceae) are also visited for the collection of that resource, which is used in the construction of nests and brood cells [67, 68]. However, *Dalechampia* flowers (Euphorbiaceae) are most commonly cited as major sources of resin for diverse species of *Eufriesea*, *Euglossa,* and *Eulaema* [20, 38, 44, 58, 69, 70]. In the present study, the only identified source of resin was a species of the genus *Dalechampia*, showing, once again, the association of Euglossini species with a species of that genus.

Analysing the pollinaria present in males collected at Picinguaba, Singer, and Sazima [71] identified 11 species of orchids belonging to nine genera. This result is similar to that shown in the present study, in which the pollinaria of ten species of Orchidaceae from eight genera were obtained. The data of the present study added to the information obtained from the region of Picinguaba by Singer and Sazima [71, 72], Pansarin et al. [73], and Pansarin and Amaral [74] increase the total numbers of Euglossini species and orchid species present in that area to 15 and 14, respectively. Only 2.13% of the 1503 males collected in Ubatuba for the present study showed evidence of association with orchids. Similarly, in the south of Minas Gerais State, Peruquetti et al. [45] found pollinaria attached to males in only 0.58% of individuals collected. These low percentages of males carrying pollinaria corroborate the observations of Ackerman [22] that such an occurrence is very uncommon in the males collected in surveys.

Although orchids are the primary sources of floral fragrances used by Euglossini males, plants of other families are also visited to obtain this resource, such as *Anthurium andraeanum*, from which males of *El. seabrai* were collected. Sazima et al. [75], also in Ubatuba, observed males of *Euglossa mandibularis* Friese, 1899 collecting fragrances in the flowers of *Solanum diploconos* (cited as *Cyphomandra diploconos*), and Soares et al. [23] recorded males of the same species in *Solanum latiflorum* Bohs (cited as *Cyphomandra calycina*) in Minas Gerais. Gracie [76] observed that male *Eufriesea convexa* (Friese, 1899) and *Eufriesea elegans* (Lepeletier, 1841) visited the flowers of *Solanum endopogon* (Bitter) Bohs (cited as *Cyphomandra endopogon* var. *endopogon*) to collect aromatic compounds. Not only the flowers but also leaves, fruits, sap, fungi that grow on rotting logs, mushrooms, and terrestrial bird droppings are also sources of aromatic compounds exploited by males [16, 77]. This information, together with the low numbers of pollinaria found in males collected in artificial baits [22], has led some authors [35, 39, 78] to question the mutualism between male Euglossini and orchids. Pemberton and Wheeler [79] showed that males of a species introduced in Florida, *Euglossa viridissima* Friese, 1899 did not depend on orchids, as they collected aromatic oils in the leaves of cultivated species such as basil, *Ocimum basilicum* L. (Lamiaceae), and allspice, *Pimenta dioica* (L.) (Myrtaceae).

The collection of floral resources from exotic plants on Anchieta Island could be a reflection of the long history of human intervention on the island, which resulted in the degradation of forest areas and the introduction of plant species of economic interest such as coconut, *Cocos nucifera* L. (Arecaceae), coffee, *Coffea arabica* L. (Rubiaceae), and sugar cane, *Saccharum officinarum* L. (Poaceae), which were cultivated during the period when the Correctional Colony was active [49].

The network formed by oil-producing flowers (Malpighiaceae) and their pollinating bees was considered by Bezerra et al. [48] as a "tiny world" within another small world of pollination networks. In the present study, the "small world" would be the study of plants visited by Euglossini bees. The connectance found in the present study was superior to large networks [80, 81] and very similar to that found in relatively small networks [82, 83]; Biesmeijer et al. [84] showed a large variation (7.2% to 37.1%) in the connectance of 27 networks of social bees and their plants.

The asymmetry of the interactions in the present study, whereby a few species have many interactions and many species have few interactions in the network, was also found by Vázquez and Aizen [85] as a trait of the system. However Bascompte et al. [86] propose that community coexistence is supported by the architecture of quantitative mutualistic networks which is characterized by the low number of strong dependences, their asymmetry, and the high heterogeneity in species strength. However, Stang et al. [87] noted that the availability of floral resources may direct interactions in a community where the population density and the amount of available flowers are also responsible for structuring the network of interactions.

The Euglossini species explored an impressive diversity of nectar sources in Ubatuba, especially plants with long, tubular corolla flowers. Flowers with corolla of this kind, which have a light colouring and produce nectar in large quantities with difficult access for floral visitors, may be classified as euglossophilous, that is, plants that have characteristics of a pollination syndrome called euglossophily [88]. Euglossini are endowed with elongated glossa and can therefore utilise a wide range of flowers, even those that have a long and narrow corolla, which are only accessible to other bees if they pierce the base, acting as robbers that are unable to pollinate the plant [35]. Roubik et al. [89] noted that Euglossini bees visit flowers that produce nectar with a high concentration of sugars to satisfy their physiological needs, because when they are active, their body temperature becomes high, requiring a high energy diet [90]. In addition to euglossophilous plants, flowers lacking tubular corolla, such as species of Fabaceae, Amaranthaceae, Lamiaceae, and Malvaceae, were also visited by bees in Ubatuba, which highlights the broad spectrum of plant species that are exploited by the Euglossini bees.

These bees are one of the most important groups of pollinators in tropical regions, not only due to interactions with different plants that produce pollen, nectar, resin, and floral fragrances but also for their role as pollinators with a wide flight radius that are able to support the reproductive biology of plants that are scattered and have low densities [11, 35]. By achieving greater diversity in tropical rain forests, which is the type of ecosystem that suffers most from the fragmentation of habitats, deforestation, and human actions [91], the Euglossini become especially vulnerable to these processes [39], which makes conservation and management essential for the preservation of these bees and their floral resources.

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Research Article

Plant Feeding in an Omnivorous Mirid, *Dicyphus hesperus*: Why Plant Context Matters

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True omnivores that feed on both plant and animal tissues are not additive combinations of herbivore and predator (carnivore). Because true omnivores must distribute adaptive feeding decisions among two disparate tissue types, understanding the context that plants provide for foraging is important to understand their role in food webs. We varied prey and plant resources to investigate the plant context in an omnivorous true bug, *Dicyphus hesperus*. The contribution of plant species to fitness was unimportant in water acquisition decisions, but affected numbers of prey consumed over longer periods. In plant communities, in the absence of prey, *D. hesperus* moved to plants with the highest resource quality. Unlike pure predators facing declining prey, omnivores can use a nondepleting resource to fund future foraging without paying a significant cost. However, the dual resource exploitation can also impose significant constraints when both types of resources are essential. The presence of relatively profitable plants that are spatially separate from intermediate consumer populations could provide a mechanism to promote stability within food webs with plant-feeding omnivores. The effects of context in omnivores will require adding second-order terms to the Lotka-Volterra structure to explicitly account for the kinds of interactions we have observed here.

1. Introduction

By definition, true omnivores (*sensu* [1]) feed at both plant and consumer trophic levels. However, these animals are not simply additive combinations of herbivores and predators (carnivores) and as such, the rules governing omnivores' use of resources might not be implied from knowledge of the two other feeding types. In addition, physical constraints (i.e., only one type of tissues may be consumed at a time) dictate that these animals must alternate foraging effort between the two types of food. If these two foods are essential, then time and food intake should be budgeted to achieve an optimum ratio of the two resources. Such diet-mixing strategies are well known for a number of herbivores [2]. If the two food types are perfectly equivalent, the omnivore should feed on whichever resource encountered [3]. If the resources are not perfectly equivalent, then the omnivore should employ some form of adaptive foraging rule that will allow one resource to substitute for the other [4, 5]. These rules can range from an increase in frequency of feeding on the less valuable resource as the more valuable declines in profitability, to a step-shaped switch in feeding activity as the profitability of the more valuable resource declines below a critical threshold. However, the rules that have been studied to date were largely those for strict herbivores and predators. It remains to be seen if such simple rules apply to omnivores, given that the aforementioned rules often lack a disparate resource context. For example, predators may choose between different resource types, but these are nutritionally relatively uniform compared to the diet choice of a true omnivore [6].

Plant feeding and prey feeding decisions have important implications for predator-prey dynamics and for energy flow within food webs [1, 7, 8]. Feeding on high-quality plant parts by omnivores can induce a partial or complete abandonment of foraging for prey, leading to outbreaks of herbivores [9]. In contrast, the increase of omnivore populations on a largely nondepleting plant resource can result in omnivore populations overexploiting prey resources and cause the extinction of those resources [7]. A decline in plant quality (profitability) can result in omnivores increasing their feeding on prey resources, and a decline in prey availability can result in an increase in feeding on plant resources [10, 11]; see [12] for an analysis of the impact of such behaviour on community dynamics. In some omnivorous true bugs (e.g., Heteroptera: Anthocoridae and Miridae), plant feeding also replaces water lost via metabolic functions [13–15] and as such, plant feeding might be considered an essential resource in some omnivore's diets.

Here, we investigate the influence of resource availability and alternate foods as contexts for plant feeding and prey feeding in an omnivore, *Dicyphus hesperus* Knight (Hemiptera: Miridae). This insect feeds on a variety of arthropod prey on several different host plants and also feeds on those host plants [16, 17]. In nature, *D. hesperus* is a generalist with respect to plant host [18] and, presumably, also to insect prey. We have observed it feeding on moth eggs, whiteflies, spider mites, thrips, and aphids in the laboratory. *Dicyphus hesperus* feeds on leaf tissue, even when prey are available [16] and relies on water obtained from feeding on leaves to replenish reserves lost to extraoral digestion [14, 15].

Prey availability and plant feeding influence correlates of fitness in this species as shown in a series of studies that we have conducted [16, 17]. Feeding on prey in the presence of leaf tissue provided an approximately 10% advantage in development time, relative to individuals provided prey with water only [16]. Reproduction and development did not differ among individuals confined to leaves of nine different host plants in the presence of prey [17]. However, in the absence of prey, these nine host plant species had different effects on both development and reproduction of this species with some plant species supporting both development and reproduction and others permitting only brief survival [17]. Taken together, these studies suggest a complex interaction between plant and animal tissue on this zoophytophagous omnivore.

In this paper, we describe a series of experiments that attempt to better understand how and why omnivores respond to disparate resources. We explore the influence of alternative resources within the foraging site (fruits, leaves, and prey) and the background of the plant community. We show that context is in fact, key to developing an omnivore feeding theory and provides some suggestions for further work.

2. Materials and Methods

Laboratory colonies were established using *D. hesperus* collected from white stem hedge nettle, *Stachys albens* A. Gray (Lamiaceae) in the foothills of the Sierra Nevada Mountains at an elevation of ca. 300 m near Woody, CA, USA (Lat. 35°42.9′ N, long. 116°49.1′ W) in 1999. These colonies were maintained at 25.0 ± 0.5°C, 23.0 ± 0.5% RH and a 16 h light (500 μE/m^2/s) and 8 h dark (0.5 μE/m^2/s) diel cycle. *Dicyphus hesperus* were reared on tobacco *Nicotiana tabacum* L. (Solanaceae) with previously frozen *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs provided *ad libitum*. These eggs were sourced from Beneficial Insectary Inc., Guelph, ON, Canada.

2.1. Selection of Plant Tissue. We start with the general observation that, in the absence of prey, *D. hesperus* feeds on tomato fruits and a blemish on the fruit is evidence of that feeding. Feeding on tomato fruits, as opposed to leaves, either confers some fitness advantages to individuals or is evidence of a change in foraging extent. In the latter case, feeding on fruit tissue might result from individuals moving from patches where prey are likely to be found (leaves) in other locations on the plant selected at random. We pursue two lines of evidence: firstly, are there fitness advantages that result from being constrained to feeding on fruit? and secondly, is there evidence that fruit tissue is selected in preference to leaf tissue?

We measured fitness as a tissue-specific function of plant feeding. We accomplished this by measuring survival and oviposition of adult female *D. hesperus* feeding on either tomato leaf or tomato fruit substrates in the presence or absence of prey (eggs of *E. kuehniella*). These experiments were conducted in small cages constructed from 250 mL Styrofoam cups. A 50 mL plastic cup (Solo) was inserted into the larger cup, and the void below was filled with tap water. For exposure to leaf tissue, the stem of single tomato leaf lobe (cultivar Patio) was inserted through a small hole into the water below. The space around the hole was filled with plastic putty to prevent *D. hesperus* adults drowning or accessing water through the opening. For exposure to fruit tissue, a green tomato fruit (cultivar Patio) was placed into the cup. A small hole in the bottom of the Solo cup was filled with plastic putty, and the void below the cup was filled with water, as in the cups with leaves. In prey treatments, *E. kuehniella* eggs were provided *ad libitum* on a 2 cm wide × 1.3 cm deep strip of Post-it note. The cages were kept at 16 h daylength and 22°C and were inspected every 2 to 3 d, and the insect state was determined (live or dead). The water reservoir was refilled and new plant and prey sources were provided at this time, and the number of *D. hesperus* eggs in the plant tissue was counted. This experiment was conducted with 20 pairs of *D. hesperus*. Males were replaced as they died. The 20 pairs were observed in three separate cohorts of 8, 7, and 5 pairs respectively. Age-specific survival and egg production were recorded. Longevity of *D. hesperus* females and total egg production were recorded from these data.

The effects of the above treatments on lifetime reproductive success were determined by calculating Euler's exact r for each cohort and treatment according to the equation:

$$1 = \sum_{0}^{\infty} e^{-rx} lxmx, \tag{1}$$

where x is time and lx and mx are the standard terms for age-specific survival and reproduction. The values of r were treated as parametric variables and analyzed by a factorial ANOVA with prey availability and plant tissue type as factors.

The effects of plant tissue type and prey availability on egg deposition were determined in a factorial design ANOVA with plant tissue type and presence/absence of prey as the factors. These data were transformed by $\ln(x + 0.33)$ prior to analysis so that the data met the assumptions of ANOVA. A Tukey HSD test was used to discriminate between treatment means. The means and 95% confidence limits (CLs) were backtransformed for presentation. The effect of treatments on longevity of adults was determined by Proportional Hazards Fit (Cox Regression) in JPM 5.1 (SAS Institute, Cary, NC, USA). The effects of plant tissue type were further analyzed by survivorship analysis within each prey-treatment regime.

If feeding on leaf and fruit plant tissue in *D. hesperus* is opportunistic, then individuals presented with the two tissues in a choice setting should express no preference for either tissue. We tested this question in Petri dish arenas (60 cm dia. × 10 cm deep) that controlled the area (amount) of fruit and leaves of tomato (CV Patio) available to starved adult female *D. hesperus*. We measured the frequency of fruit feeding, based on the number of blemishes accumulated on the tomato fruit disc in 24 h. Because feeding on leaf tissue leaves no blemishes or other quantifiable evidence, we were constrained to assess leaf feeding effort indirectly. Fruit and leaf discs were offered in two areas, 50 mm^2 or 12.5 mm^2, and choices were presented as 50 mm^2 pairs, or 12.5 mm^2 versus 50 mm^2 unmatched pairs. Two fruit discs presented together, 50 mm^2 each, provided a measure of fruit feeding frequency when no leaf resource was available. The leaf discs were obtained from young, fully expanded tomato leaves using a cork borer with a 65 mm^2 opening and were cut to avoid major leaf veins. Fruit discs were obtained by using the same cork borer to extract a core from the equatorial plane of green tomato fruits then cutting away the tissue below the margin of the perimeter of the 65 mm^2 disc of epidermis and fruit tissue. The appropriate size was then produced in the arenas using masks of Glad Press'n Seal (The Glad Products Company, Oakland, CA, USA), in which openings of the appropriate sizes were cut. Observation showed that this produced a seal around the perimeter of the plant tissues, and that adult *D. hesperus* were unable to feed through this material. If fruit tissue provided an equal resource to leaf tissue, then the number of blemishes on the fruit should be in proportion to its relative availability in the arena. We calculated a predicted number of blemishes on fruit in each type of arenas by multiplying the number of blemishes present when only fruit tissue was available by the proportion of fruit tissue in the arena. We then subtracted the predicted blemishes from the observed blemishes and, for each proportion of fruit, determined if this difference was different from zero by a Wilcoxon signed-rank test (JMP 7.0).

2.2. Relative Effort of Feeding on Plant and Prey Resources. Omnivores can use the disparate resources in their diet in two fundamentally different ways. They can diet-balance, and thus acquire the two disparate resources in proportions that provide an optimum diet. Alternatively, they can forage adaptively and only feed on the less valuable resource in the absence of the more profitable resource. Previous work on *D. hesperus* suggest that this insect should diet-balance, since it is dependent on water from plants for production of saliva, and thus for extraoral digestion of prey tissue [14, 15]. Plant sap in the diet confers a slight development time advantage compared to individuals provided only water from a wick [16]. Some plant species support development and reproduction of *D. hesperus* and others do not [17].

We used the time allocated to plant and prey feeding following deprivation of these resources to examine the hypothesis that *D. hesperus* uses a diet-mixing strategy to allocate effort to feeding on plant and prey resources. We conducted these experiments on three plant species that have been previously demonstrated to have different profitabilities for *D. hesperus*. We provided prey (*E. kuehniella* eggs) together with one of three plant species for 24 h, followed by 24 h provision of both, either or neither of the resources. The effect of these treatments was subsequently measured by observing the time devoted to plant and prey feeding in a subsequent 2 h observation where both resources were provided. If *D. hesperus* used a diet-mixing strategy in foraging, then we predicted individuals would subsequently allocate time to foraging on the resource that had been absent during treatment. If the profitability of tissue from different plant species affected foraging decisions, then plant species should affect the effort allocated to foraging.

Freshly emerged (<48 h old) adult females were extracted from rearing cages. These were identified by the light coloration of the wings and green coloration of the abdomen. Insects were placed in 50 mL plastic cups (Solo Cup Corporation) with eggs of *E. kuehniella* supplied *ad libitum* on a strip of Post-it note (3 M Corporation, Minneapolis, MN, USA), and a leaf of either chrysanthemum, *Chrysanthemum coronarium* L. (Asteraceae), tomato, *Lycopersicon esculentum* Mill cultivar Rhapsodie (Solanaceae), or mullein, *Verbascum thapsus* L. (Scrophulariaceae). The petioles of the leaves protruded through a hole in the bottom of the cup into a water reservoir below, in order to keep the leaves in fresh condition. These were held in the laboratory at 22°C, with room lighting and a daylength of 12 h. This preexposure ensured that the *D. hesperus* had experience with the plant and prey combination, and that all females within a plant species and prey group were in similar states when the treatment period began.

After 24 h, the insects were transferred from cups containing both leaves and prey to cups containing the experimental treatments. Treatments were without prey/without plant; without prey/with plant (a leaf of the same species as provided in pre-exposure); with prey (*E. kuehniella* eggs *ad libitum* on a Post-it note strip)/without plant; with prey/with plant. As previously, cups with plants had leaf pieces with petioles protruding through the bottom of the cup into a water reservoir, which prevented wilting. These were held for 24 h on a benchtop in the laboratory.

The effects of plant and prey deprivation treatments on the within-leaf feeding responses of individual *D. hesperus* adults were evaluated in arenas constructed from 50 mm plastic Petri plates. Notches were cut in the edges of the bottom and top halves to accommodate leaf petioles. Leaves of chrysanthemum, tomato, and mullein were cut to fit the inside of the dish. The petiole of each leaf piece was extended outside the dish through a small slot. The petiole was placed into a vial of water to prevent wilting during observation. Prey were supplied in all arenas (50 *E. kuehniella* eggs on a Post-it note strip) and these were placed onto the leaf surface. Insects were moved from the treatment cups into evaluation arenas containing the same plant species as that on which they had been preexposed and treated.

Dicyphus hesperus adults were observed continuously for 2 h. The start and finish times of bouts of plant feeding and prey feeding were recorded with the aid of a stopwatch. Times spent in plant and prey feeding during the entire observation period were calculated by summing the feeding times. A complete set of evaluation trials (including all three plants species and all four deprivation treatments for each) was recorded by two observers (six arenas per observer), and plant species and deprivation treatments were assigned haphazardly to observers. Sinia [19] demonstrated that time spent in feeding and weight of food consumed were highly correlated in *D. hesperus* thus; we, did not weigh prey or plants to determine the mass consumed. The experiment was repeated 15 times.

The effects of host plant species (plant species), access to plant material during treatment (plant deprivation) and access to prey during treatment (prey deprivation) on the times spent in plant feeding and prey feeding during subsequent observation were analyzed in a factorial design, three-way MANOVA (response = contrast) in JMP 7.0. A multivariate approach was required because prey feeding and plant feeding are mutually dependent within subjects, that is, the test subject can only do one thing or the other at any given time. Moreover, many subjects only fed on one resource, meaning that the dataset contained extreme values at both ends of the distribution, and data transformations could not produce a normal distribution. Females that fed on neither resource were not included in either analysis.

2.3. Effects of Plants Species on Predation. We evaluated the effects of plant species on predation. Because omnivores like *D. hesperus* feed on plants to acquire water [14, 15], plant species and their associated differences in biochemistry may not actually provide a context for feeding decisions and foraging effort. Sanchez et al. [17] showed that, in the presence of prey, plant species did not affect development or reproduction so, plants may simply be a source of water and not affect foraging effort or foraging decisions. We evaluated the effects of plant species on prey feeding activity following periods of starvation. The purpose was to determine if plant feeding affected the level of hunger, and thus reduced prey feeding when prey subsequently became available. Female *D. hesperus*, 7 or fewer days old, were placed in small cages with no prey, and a leaf of either chrysanthemum, mullein, pepper, tomato, or a water wick. These females were held, without prey, for 0, 1, 2, 3, 4, 5, or 6 days of starvation. Leaves were replaced if they degraded. Prey (eggs of *E. kuehniella*) were then provided in the cages, on 1 cm wide pieces of Post-it Note, as above, for 7 hours. The number of eggs that were consumed was counted. The data were transformed by $\log 10(x + 1)$ to correct for lack of normality, and analyzed by an analysis of covariance model, using JMP 10.0 (SAS Institute, Cary, NC, USA).

Plant community potentially provides a different context for foraging from that of individual plants. Sanchez et al. [17] showed that life history and reproduction of *D. hesperus* did not differ across plant species in the presence of prey, suggesting that the availability of water to sustain extra-oral digestion may not differ across plant species. However, plants can provide other resources including nutrition, shelter and refuge, and innate expectation of prey. We evaluated the effect of plant community on foraging effort by female *D. hesperus*. Female *D. hesperus*, approximately 7 days old, were starved in 500 mL cup cages, with a tomato leaf, for 48 h, then placed in 65 cm cube cages with a tomato plant ("Rhapsodie," Rodgers Seeds, Boise, ID, USA) and one other plant species—either tomato, mullein or chrysanthemum. Four Postit note strips, each with abundant (>1000) *E. kuehniella* eggs were placed on each leaf of the tomato plant. After five days, we counted the numbers of eggs consumed on each strip and relocated the female. We considered the effects of plant community on two variables: the total number of eggs eaten and the number egg patches visited on the tomato plant. The former was analyzed by a least squares ANOVA. Egg count was transformed to $\log 10(x + 1)$ to correct for lack of normality. The number of visits was analyzed by logistic regression. The experiment was repeated 48 times for each alternate plant species, but we only analyzed data for cages where the female could be relocated at the end of the experiment.

2.4. Adaptive Foraging in the Presence of Prey. Experiments described above demonstrated that *D. hesperus* does not exhibit a preference for tomato fruit tissue over tomato leaf tissue, although being constrained to long-term feeding on tomato fruits in the absence of prey did confer a slight advantage in survival in females compared to females constrained on leaf tissue. In order to demonstrate that feeding blemishes on green fruits on whole plants indicate a change in foraging behaviour that is dependent on the profitability of available resources, we conducted the following experiment. Tomato plants, (cultivar Patio), 12 weeks old, in a peat-based potting mix, in 15 cm pots, were reduced to 4 leaves and 4 green fruit. These were placed in 65 cm by 65 cm cages that were covered with fine cloth. Eggs of *E. kuehniella* on 1 cm wide × 1.3 cm deep Post-it note strips served as prey patches. Three prey treatments were used: high prey, consisting of a patch of >1000 eggs on each leaf; a low prey treatment consisting of a patch of approximately 50 eggs on each leaf; a zero prey treatment. Five female *D. hesperus*, <7 days old, were placed in each cage. After 7 days, the fruit were removed from the plants and examined for feeding punctures, which were counted and pooled across fruit within cages. The experiment was replicated 10 times. Prey availability treatments were compared using a one-way ANOVA, and means were separated using a Tukey test with $\alpha = 0.05$.

Table 1: Mean (upper and lower 95% CL) total eggs laid, lifetime (upper and lower 95% CL) and mean ± SEM, Euler's exact r for female *D. hesperus* on either tomato leaf or tomato fruit, with or without prey (eggs of *E. kuhniella*). $N = 20$.

	Total eggs laid ($N = 20$ females)	Longevity ($N = 20$ females)	Euler' exact r ($n = 3$ cohorts)
Fruit, without prey	1.3 (0.8, 1.9)	17 (10, 23)	0.98 ± 0.023
Fruit, with prey	15.0 (11.8, 19.0)	31 (15, 39)	1.08 ± 0.043
Leaf, without prey	3.0 (2.3, 4.0)	6 (3, 6)	1.21 ± 0.023
Leaf, with prey	41.8 (27.6, 63.1)	28 (4, 50)	1.34 ± 0.037

2.5. Adaptive Foraging for Plant Resources. In the absence of prey, and opportunities to diet-balance, omnivores should feed on the most profitable plant resource available to them within the plant community. We tested this hypothesis using feeding punctures on green tomato fruits on a tomato plant as the indicator of feeding on various resources within cages and in greenhouses. In cage experiments, tomato plants (cultivar Patio) with fruit as above were paired with one of the following plant treatments: a single mullein plant, a single chrysanthemum plant, a single tomato plant (cultivar Rhapsodie), or no other plant. There were no fruit or flowers on any of these alternative plants. Ten females were placed in each cage. The cages were inspected daily and the numbers of insects on the Patio tomato plant was counted. The tomato fruit were harvested and feeding punctures on the fruit counted after 7 days. The sum of the numbers of *D. hesperus* observed on the Patio tomato plants in each cage over 7 days served as an index of the effects of plant community on potential for feeding on tomato fruits by *D. hesperus*. Our *a priori* hypothesis was that the presence of any second plant in the cage would reduce the number *D. hesperus* on the tomato plant by approximately half, and that this would result in a similar reduction in the amount of blemishing on the tomato fruits. There were 7 replicates each of the "none" and "Rhapsodie tomato" treatments and 8 each of the "mullein" and "chrysanthemum" treatments. One replicate of the "none" treatment was lost due to blossom end rot on the fruits. Blemish data were transformed by log base 10 to correct for dependence of the variance on the mean. Backtransformed means and SE values are presented. The effects of prey treatment on numbers of insects on plants and on feeding punctures on fruit were compared using a one-way ANOVA, and means were separated using a Tukey test with $\alpha = 0.05$.

A greenhouse experiment was conducted to further examine the effects of plant community on plant feeding in the absence of prey. Tomato plants, CV Rhapsodie, were grown in hydroponics culture in 4 glass, greenhouse compartments (3.2 by 12 m). These compartments were each split into two, 3.2 by 6 m enclosures with a curtain of Agryl P17 spun bond row cover (BBA Fiberweb, London, UK). There were ten plants in each enclosure, arranged in two rows of five each. These plants had grown to the full height of the trellis wire (3.35 m), and each plant bore approximately 8 trusses of fruit ranging in age from freshly pollinated to near-ripe. There were no prey on any of the plants. In one enclosure in each compartment, a single mullein plant, with a rosette diameter of approximately 60 cm, in a 30 cm hanging basket pot, was placed in the centre of the plants, suspended within the tomato crop canopy. Fifteen *D. hesperus* were released on each tomato plant. The release population consisted of 3 males, 3 females, 3 large nymphs, and 6 small nymphs, which approximated the population age structure in a previous experiment [17]. The numbers of insects on each plant was counted three times during the experiment, on days 3, 7, and 11. After 14 days, all fruit were removed from all trusses on all plants in each house, and the number of feeding punctures on each fruit was counted. Data were recorded by plant truss, numbered from lowest to highest on the plant, in order to also determine if position of fruit on the plant affected the likelihood of that fruit being blemished. Effects of the presence of mullein on numbers of *D. hesperus* on plants were determined by repeated measures (RMs) ANOVA. Effects on the proportion of fruit with feeding punctures were determined by 2-way ANOVA on arcsin (square root x) transformed data, with mullein presence-absence (treatment) and truss number as main effects. Summary data on proportion of blemished fruits are reported as backtransformed lower 95% CL < mean > upper 95% CL.

3. Results

3.1. Selection of Plant Tissue. The intrinsic rate of increase, r, was lower when females were provided fruit tissue than when provided leaf tissue ($F_{1,8} = 54.3939$, $P < 0.0001$, Table 1) and was higher when prey were provided than when not ($F_{1,8} = 12.8684$, $P = 0.0071$). There was no interaction between the factors ($F_{1,8} = 0.2236$, $P = 0.6489$). Female *D. hesperus* laid fewer eggs when on fruit than on leaf tissue ($F_{1,76} = 7.63$, $P = 0.0072$) and more eggs when given prey than when deprived ($F_{1,76} = 57.35$, $P < 0.0001$), and there was no interaction between the factors ($F_{1,76} = 0.19$, $P = 0.6625$) (Table 1). There was an interaction between plant and prey with respect to overall longevity (L-R $\chi^2 = 7.03$, $P = 0.0080$). Therefore, the effect of plant tissue type was analyzed within prey treatment. In the absence of prey, females on fruit lived longer than females on leaf tissue (L-R $\chi^2 = 26.23$, $P < 0.0001$) and in the presence of prey there was no difference (L-R $\chi^2 = 0.14$, $P = 0.7116$). Thus, feeding on fruit tissue in the absence of prey confers a slight advantage in longevity over feeding on plant tissue. There is a disadvantage to feeding on fruit tissue in the presence of prey.

3.2. Plant Tissue Preferences. In Petri dish arenas with different proportions of leaf and fruit tissues available, the

Table 2: Blemishing by *D. hesperus* females on fruit disks of different sizes in Petri dish arenas with different combinations of leaf and fruit tissue available. The observed blemishes on fruit in arenas containing two different tissue types were subtracted from the area-adjusted prediction for blemishes from the arenas with only fruit tissue, and tested by Wilcoxon signed-rank test to determine if this difference deviated from zero. $N = 30$ for all tests.

Fruit area (mm^2)	Leaf area (mm^2)	Blemishes per fruit disk	Wilcoxon test result
100	0	3.57 ± 2.24	—
50	50	1.17 ± 2.08	$P = 0.0054$
50	12	2.96 ± 3.07	$P = 0.44$
12	50	0.87 ± 1.59	$P = 0.33$

Table 3: Results of a three-factor MANOVA (response = contrast) of time spent in plant feeding and time spent in prey feeding by *Dicyphus hesperus* females.

Factor	MANOVA results		
	df	*F*	*P*
Intercept	1, 120	30.50	<0.0001
HOST	2, 120	0.65	0.5237
PLANT	1, 120	10.76	0.0014
PREY	1, 120	26.97	<0.0001
HOST∗PLANT	2, 120	0.53	0.5924
HOST∗PREY	2, 120	0.96	0.3846
PLANT∗PREY	1, 120	7.65	0.0066
HOST∗PLANT∗PREY	2, 120	0.84	0.4334

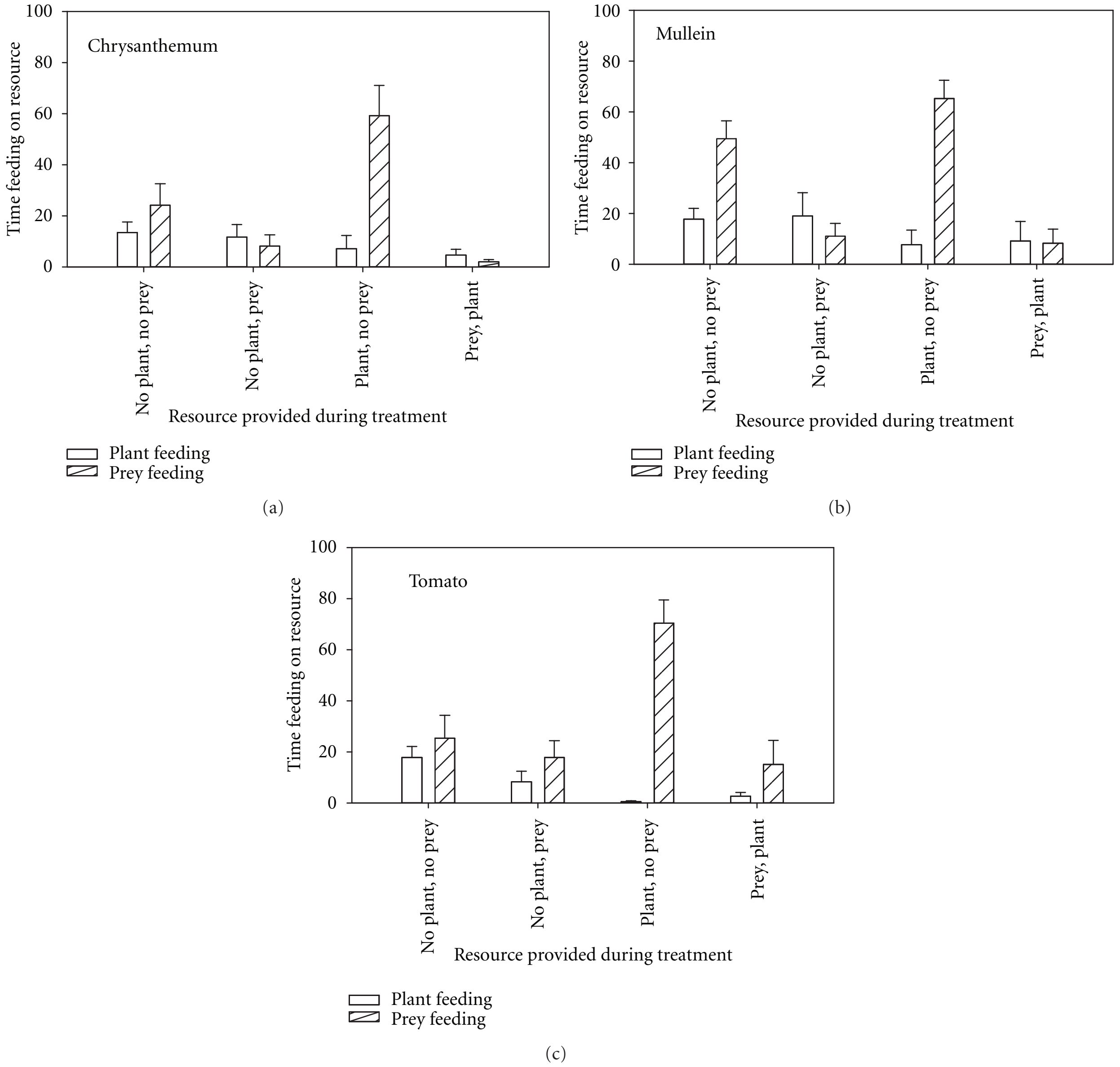
number of blemishes on the fruit discs was less than expected in arenas with an equal proportion of leaf and plant tissues (Table 2). Otherwise, the number of feeding blemishes on fruit tissue was not different from the number expected. This result suggests that when the two tissue types were equally available, *D. hesperus* females fed more frequently on leaf than fruit tissue.

3.3. Relative Effort of Feeding on Plant and Prey Resources. The resources provided to *D. hesperus* females during the experimental period had a significant effect on the time devoted to feeding on either of the two resources (Table 3). When deprived of prey or plant prior to full access, females spent more time feeding on the deprived resource than when it had been available during the experimental period (Figure 1). There was an interaction between plant and prey access during the experimental period. Females that were deprived of prey, but provided plant, spent relatively less time plant feeding than females in other deprivation treatments (Figure 1). Host plant species did not affect the relative time spent feeding on plant and prey resources following the deprivation period. Thus, there is evidence that *D. hesperus* diet-balances by expending effort to replace the resource that has been deprived.

3.4. Effects of Plants Species on Predation. Plant species affected the way in which female *D. hesperus* responded to prey in starvation treatments (Figure 2, analysis of covariance, Plant host ∗ days of starvation, $F_{4,259} = 2.76$, $P = 0.0281$). The number of prey consumed increased with starvation period for insects confined to pepper, tomato, or water wicks (linear regression, $F_{1,51} = 13.35$, $P = 0.0006$; $F_{1,62} = 10.29$, $P = 0.0021$; and $F_{1,58} = 4.63$, $P = 0.0357$, resp.) whilst prey consumption remained constant over time for insects confined on chrysanthemum and mullein (linear regression, $F_{1,59} = 0.66$, $P = 0.042$; $F_{1,54} = 0.56$, $P = 0.46$, resp.). The average number of prey consumed during foraging bouts was affected by plant species (analysis of covariance, $F_{4,259} = 5.09$, $P = 0.0006$, and Tukey HSD). The number of prey eaten by female *D. hesperus* was significantly greater for insects confined on chrysanthemum (1.47±0.037) than those confined on mullein (1.13 ± 0.074) and tomato (1.22 ± 0.067). The overall numbers of prey consumed on pepper (1.22 ± 0.075) and the water wick (1.35 ± 0.051) were not different from each other or from the extremes.

In the plant community experiment, the species of the alternate plant affected the number of prey eaten ($F_{2,114} = 5.94$, $P = 0.0014$). More prey were eaten when the alternate plant was chrysanthemum (193 ± 28.5, $N = 41$) than when it was tomato (75 ± 1.2, $N = 38$). The number of prey eaten when mullein was the alternate plant (121 ± 25.0, $N = 38$) was not different from either extreme. The number of prey patches that were attacked was affected by the species of the alternate plant (logistic regression, log-likelihood Chi Square = 17.9, df = 2, $P = 0.0001$), and more visits were made to the prey patch when chrysanthemum was the alternate plant that when mullein or tomato were the alternate plants. The females visited the prey patch 2.0 ± 0.21 times when chrysanthemum was present, 1.2 ± 0.17 times when mullein was present, and 0.9 ± 0.15 times when tomato was the alternate plant.

3.5. Adaptive Foraging for Plants in the Presence of Prey. The presence of prey on the leaf reduced the number of feeding



(a)

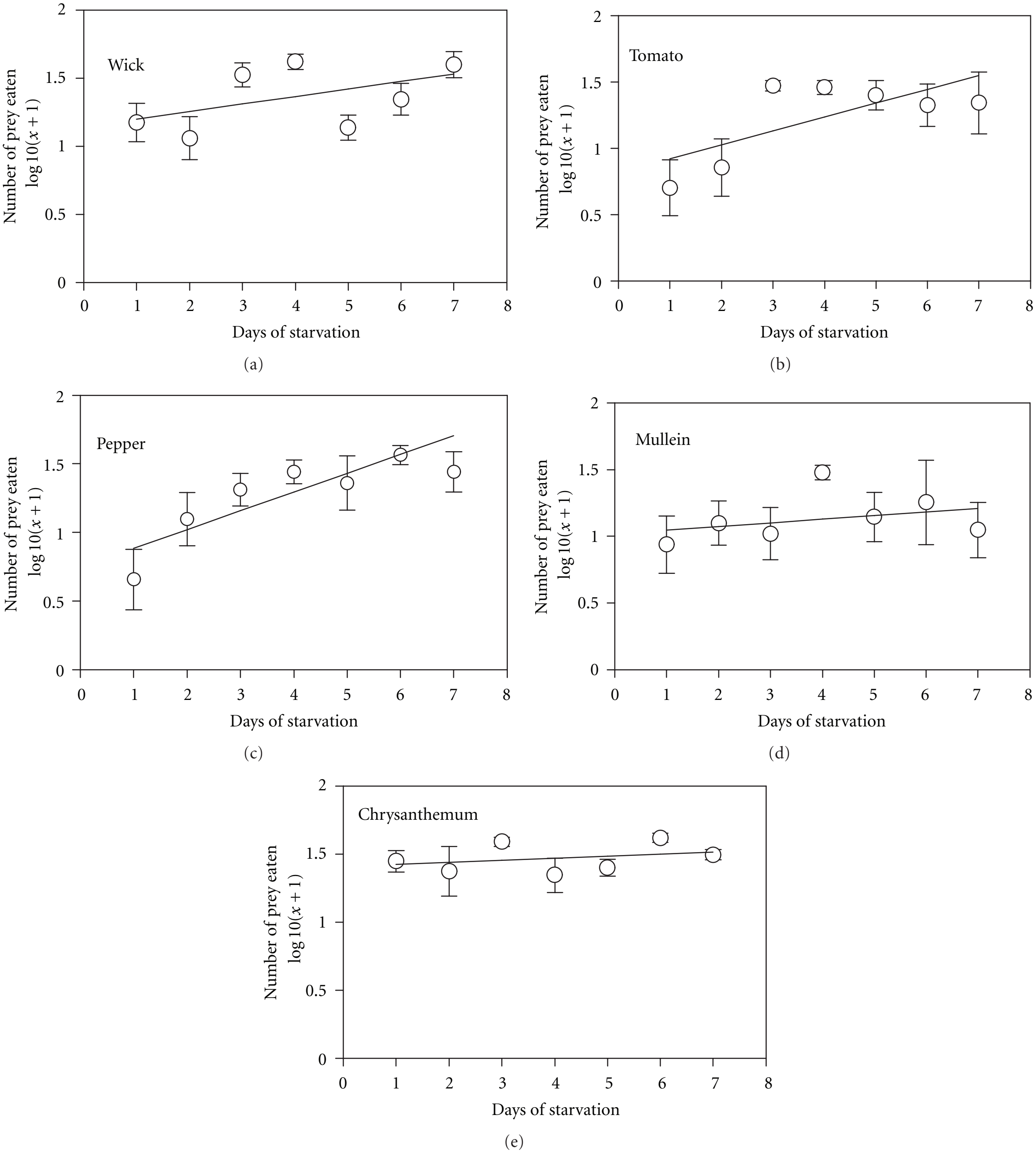
(b)

(c)

Figure 1: Mean total time spent in (a) plant and prey feeding during a 2 h observation interval and (b) proportion of feeding time that was plant feeding by *Dicyphus hesperus* females after 24 h experimental periods with or without access to plant tissue and/or prey.

punctures on fruit on whole tomato plants ($F_{2,27} = 8.12$, $P = 0.0017$). There were more feeding punctures on fruit in cages without prey (46.8 ± 14.15) than on fruit in cages with low prey availability (3.9 ± 1.42) or high prey availability (5.6 ± 2.65), which were not different from each other (Tukey test, $\alpha = 0.05$). Thus, *D. hesperus* forages adaptively, for the most valuable resource, and the presence of blemishes on green fruit represents a shift in foraging strategy. Interestingly, the presence of even very small numbers of prey on plants was sufficient to keep the insects from shifting to feeding on fruits.

3.6. Adaptive Foraging for Plant Resources. In cage experiments examining the effects of plant community on adaptive foraging for plant resources, experimental run was not a significant effect for either accumulated numbers of *D. hesperus* or numbers of blemishes on fruits ($F_{1,22} = 2.266$, $P = 0.080$; $F_{1,21} = 0.403$, $P = 0.533$). Therefore, further analysis was done without this factor. Blemishing results for one cage were discarded due to disease symptoms on fruit. The accumulated numbers of *D. hesperus* were highest on patio tomato plants paired with no alternative plant, intermediate on Patio tomato plants paired with either chrysanthemum or tomato cultivar Rhapsodie, and lowest on Patio tomato plants paired with mullein (Table 4; $F_{3,26} = 21.722$, $P < 0.001$). Feeding punctures on tomato fruits, however, did not follow the same pattern, and feeding punctures were lowest on patio tomato plants paired with mullein and not different among the remaining combinations (Table 4, $F_{3,25} = 9.611$, $P < 0.001$). These results indicate that *D. hesperus* uses



(a) (b) (c) (d) (e)

Figure 2: Number of prey consumed by female *D. hesperus* after different periods of starvation while confined to chrysanthemum, mullein, pepper, or tomato leaves, or with a wick providing water. *N* varies from 10 to 3 for each point.

an adaptive foraging strategy for plant species in communities and that it spends time in and devotes plant feeding effort to the plant resource that provides the best fitness returns.

In greenhouse experiments, the number of *D. hesperus* on Rhapsodie tomato plants in greenhouses decreased over time (Figure 3; RM ANOVA, $F_{2,5} = 19.90$, $P = 0.004$), but there was no effect of treatment on the number of insects in greenhouses (RM ANOVA, $F_{2,5} = 1.12$, $P = 0.396$). There was a difference in the proportion of blemished fruit (ANOVA, $F_{1,9} = 8.19$, $P = 0.0059$); the proportion of fruit blemished was $0.012 < 0.023 < 0.038$ in greenhouses with mullein and $0.020 < 0.050 < 0.078$ in greenhouses without. Variance values are backtransformed confidence limits, which are presented because the arcsin (squareroot X) transformation yields asymmetric values for variance. The proportion of blemished fruit varied with truss number (Figure 4, $F_{1,9} = 4.81$, $P < 0.001$), but there was no interaction between treatment and truss with respect to the

Table 4: Mean ± SE (N) accumulated numbers of *D. hesperus* on Patio tomato plants and number of blemishes on fruit in the presence of different alternative plant species. Means in columns followed by the same letter are not significantly different (Tukey HSD, $\alpha = 0.05$).

Alternative plant	Accumulated *Dicyphus*	Number of blemishes on Patio tomato
None	50.3 ± 3.25 a (7)	67.9 ± 5.88 a (7)
Chrysanthemum	35.4 ± 3.61 b (8)	51.2 ± 16.92 a (8)
Tomato (cultivar Rhapsodie)	27.4 ± 2.8 b (7)	43.3 ± 15.70 a (7)
Mullein	12.7 ± 2.7 c (8)	6.2 ± 2.16 b (8)

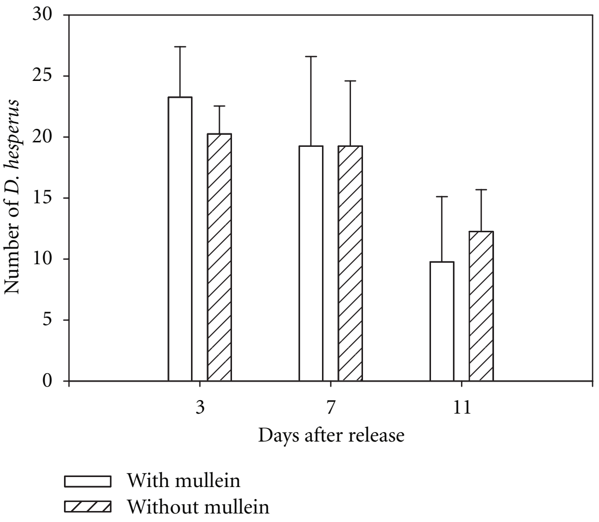


Figure 3: Number of total *D. hesperus* (all stages) on tomato plants in greenhouses in the presence or absence of mullein banker plants and the absence of prey. Values are means ± SE, and $N = 4$ for each bar.

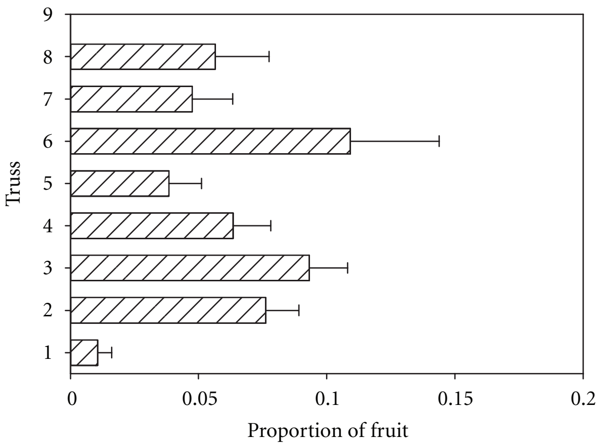


Figure 4: Distribution of blemishing on tomato fruits at different heights (truss 1: low and most mature, truss 8: high and most immature) on tomato plants 14 d after release of *Dicyphus hesperus*' adults and nymphs.

proportion of fruits blemished ($F_{1,9} = 1.07$, $P = 0.3958$). Fruits on the lowest (most ripe) truss were attacked at a lower frequency than other fruits on the plant. These results provide further evidence for the apparent adaptive plant foraging strategy evident in the small cage experiments.

4. Discussion

In the introduction, we posited that true omnivores are not simple, additive combinations of herbivore and predator. As such, both the plant and prey environment should provide context for foraging behaviour and should influence this behaviour in ways that are unique to animals that have an explicitly omnivorous diet. Prey and plant foraging decisions should depend on the specific identity of both the plant and prey resource, and evidence for this context should emerge from the statistical interactions between plant and prey factors in experiments. In fact, our results reveal exactly these interactions and demonstrate that, for true omnivores like *D. hesperus*, the plant context is extremely important. In the discussion that follows, we explore this hypothesis and its implications for arthropod plant dynamics in food webs containing true omnivores.

We assume that true omnivores forage for plant and prey resources according to the rules of optimal foraging theory. Thus, omnivores should forage to maximize fitness and can do so by balancing diet, patch type, or time allocation [20]. A central concept of optimal foraging theory is that, for any finite resource, food intake in a resource patch should decrease with increasing time in the patch due to patch depletion [21]. Patch depletion has an important influence on the evolution of optimization decisions. However, for small, plant-feeding omnivores that inhabit plants and feed on plant tissue (as opposed to pollen or nectar) the plant resource is essentially unlimited and does not readily deplete with feeding. Optimal foraging decisions in these organisms could therefore be made based on depletion of the prey resource and the potential contribution of proximate plant quality to fitness (profitability). In this work, we have not attempted to calculate the optimum behaviours for *D. hesperus* and we use "optimum" in the sense of seeking the best decision providing the highest fitness returns in a qualitative, rather than quantitative sense. Evidence for these relative fitness returns can be garnered from our experiments here and from previous studies examining the effects of plant and prey diet on correlates of fitness [14, 16, 17].

For *D. hesperus*, the relative contribution of prey species (animal tissue) to fitness is quite similar when contrasted with the contribution of plant tissue [17], and in general, the differences among plant tissue are of greater relative magnitude than differences among prey tissues (e.g., [16, 17] cf. [6]).

In the absence of prey, *D. hesperus* foraged on tomato fruits, which led to an increased lifespan. This might increase the opportunity for the insect to locate additional resources that would support reproduction. However, being constrained to feed on tomato fruit tissue significantly reduced the estimated intrinsic rate of increase, both with and without prey. Therefore, feeding exclusively on tomato fruits could be detrimental to *D. hesperus*. The intrinsic rate of increase is an estimate of the potential profitability of a resource [22] because it measures the effects of the resource on potential population growth. In nature, *D. hesperus* would not be constrained to feed continuously on a resource like tomato fruits, and the short-term gains in longevity might outweigh any incremental losses in reproductive potential if they increase the probability of being able to find prey in the future. In contrast, a pure predator facing declining prey resources does not have the opportunity to use its sole resource (prey) to fund future foraging returns without paying a significant cost. When such resources are in decline, then investment in energy stores would necessarily tradeoff with somatic and gametic investment.

Above we discussed the dual resource exploitation as an opportunity for omnivores; however, such a feeding strategy can also impose significant constraints when both types of resources are essential. When deprived of either plant or prey resources, *D. hesperus* females increased the time feeding on the deprived resource, suggesting that a degree of diet balancing was occurring. However, plant species did not affect the time spent plant feeding. Presumably, *D. hesperus* were primarily replenishing water reserves, and this result suggests that all plant leaf tissues provide similar access to water. This is consistent with the results of Sanchez et al. [17]. The relative time spent in plant feeding across all deprivation treatments was considerably longer than time spent in prey feeding, which reflects the relative contribution of the two resources to fitness.

Based on the lack of effect of plant species on prey feeding following deprivation, we expected that plant species should not affect prey consumption over longer periods. However, plant species did affect prey consumption in *D. hesperus* following longer periods of prey deprivation. When starved for up to 7 days on chrysanthemum, *D. hesperus* ate more prey, when these were provided, than when starved on mullein. Mullein has been shown to provide sufficient nutrition to sustain development and reproduction in *D. hesperus* whereas chrysanthemum does not [17]. Because prey were provided on a common substrate, it is unlikely that plant surface characteristics affected prey consumption. In longer-term consumption experiments in simple plant communities, *D. hesperus* ate more prey and visited more prey patches in communities with chrysanthemum and tomato than in communities with tomato only, reflecting greater effort devoted to foraging when a low-quality plant was present. Overall, the number of prey consumed by *D. hesperus* was determined by hunger, by plant substrate, and by plant community.

The makeup of plant communities also influenced herbivory in *D. hesperus*. In both cage and greenhouse experiments, herbivory on tomato plants in the absence of prey, as measured by blemishing on fruits, was determined by the makeup of the plant community. In particular, the presence of mullein reduced blemishing, relative to monoculture tomato communities and ones with chrysanthemum. This is consistent with the above experiments, which suggest that plant contribution to fitness should be part of adaptive foraging decisions in *D. hesperus*.

Studies on other omnivores foraging for spatially overlapping, complementary resources (e.g., [23]) suggest that as the availability of one resource declines, a dietary shift to the other resource should occur. Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), reduce feeding on cotton leaves in the presence of spider mites, *Tetranychus pacificus* (Acari: Tetranychidae), and this reduction appears to be a response to reduction in plant quality through induction of plant defense by spider mite feeding on the cotton plant [24]. On poor-quality plants, reproduction and survival of *F. occidentalis* increased as a result of feeding on predator eggs, but on high-quality plants omnivorous feeding had no effects [25]. Plant feeding on cotton by *Geocoris punctipes* occurred in the presence of both high- and low-quality prey, but was greater in increase in the presence of the poor-quality prey [22].

Other omnivorous insects strategically shift feeding efforts based on availability of plant and prey resources. In the presence of high-quality fruit (pods) on soybeans, *Geocoris pallens* reduced predation on aphids [26]. The availability of pollen (a high-quality plant food) to omnivorous mites can reduce predation on thrips and change the distribution of predator mites on leaves [27]. *Dicyphus tamaninii* feed on tomato fruits in the absence of prey [10, 28], but in this species, development of nymphs will occur on tomato fruits in the absence of prey [29]. However, in *D. hesperus*, feeding on tomato fruits may not be an adaptive foraging strategy, if there is no profitability associated with the resource. In addition, *D. hesperus* expresses no preference for tomato fruit tissue over leaf tissue. Therefore, other explanations must be sought for the observed feeding on tomato fruits in the absence of prey. Patch abandonment in *D. hesperus* is influenced by both plant and prey profitability [30]. In our cage experiment, *D. hesperus* could not abandon the patch, since there were no other plant resources in the cage, and plant feeding is essential for survival. The distribution of *D. hesperus* is likely determined by the distribution of its prey, since, at least on tomato plants, the distribution should not be influenced by within-plant differences in plant tissue profitability. Therefore, in the absence of prey it is possible that *D. hesperus* forages for prey on all plant parts. Feeding on fruit tissue may be the result of the insects opportunistically engaging in herbivory while on fruits, as opposed to deliberately locating themselves on fruits in order to feed. Alternatively, domesticated tomatoes may not confer the same benefits as wild solanaceous plants, but they might provide the same gustatory cues meaning *D. hesperus* may be caught in an ecological trap. The plant and prey communities used in these experiments are quite artificial and derive from our previous work on the application of this species as a biological control agent.

Where plants in communities vary in their direct contribution to fitness and in their actual or potential prey content, omnivores should utilize resources in those communities according to optimal foraging rules, and thus plants should provide context for feeding behaviour in omnivores. Omnivorous insects are known to respond to plant species or quality at the community scale in the presence or absence of prey. Western flower thrips were more prevalent, during migration, in flowers of tomato plants with a high nitrogen status, compared to those with low N status [31]. *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) forages for a native geometrid on a native plant, guava (*Psidium guajava* L.), in preference to the same prey species on the exotic *Eucalyptus cloesiana* (F. Muell), despite high prey abundance on the latter [32]. Populations of *Orius insidiosus* in fields were higher on bean and corn than on pepper or tomato [33]. The former two crops support development, longevity, and reproduction to a larger degree than the latter two crops [34, 35]. For *D. hesperus*, plant profitability similarly determines where the insects will be located and on what they will feed. This is determined by emigration driven by prey and plant profitability and by the profitability of other available plants in the community (immigration).

In a broader context, true omnivores, that is animals that feed on both plant and prey, use plants in a number of ways, depending on the species and situation. Feeding on plant tissue can top-up prey feeding, can replace prey feeding, or can provide essential nutrients that are required as part of an omnivore diet. The foraging strategies employed by true omnivores to optimize the use of plant and prey resources clearly depend on a plant context. True omnivores that live on plants may feed on the intermediate consumer (e.g., herbivorous prey) available on the plant, can feed directly on the plant part on which prey occur (e.g., leaves), seek out other tissue within the plant, such as fruits, growing points or seeds, or move to other plants or plant species within the community. The stability of communities containing omnivores has been a persistent theme in ecology over the past decade (e.g., [4, 5, 7, 8, 36]), and several theoretical mechanisms have been explored that might promote stability. Křivan and Diehl [5] proposed that stability should be promoted when an omnivore forages adaptively, and the intermediate consumer is the more profitable prey. As the intermediate prey number declines, the omnivore either switches to feeding on the less profitable prey, or broadens its diet, thereby providing the intermediate prey with a refuge from predation. For *D. hesperus*, prey are far more profitable than plants [16, 17] and plant species vary considerably in their profitability [17]. It seems likely that as prey decline in number, and thus patch value declines, the presence of relatively profitable plants that are spatially separate from those supporting intermediate consumer populations would provide a mechanism to promote stability. We suggest that exploring the effects of the context of plants for foraging decisions in *D. hesperus* will require adding second-order terms to the standard Lotka-Volterra structure in order to explicitly account for the kinds of interactions we have observed here.

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Research Article

Flight Dynamics and Abundance of *Ips sexdentatus* (Coleoptera: Curculionidae: Scolytinae) in Different Sawmills from Northern Spain: Differences between Local *Pinus radiata* (Pinales: Pinaceae) and Southern France Incoming *P. pinaster* Timber

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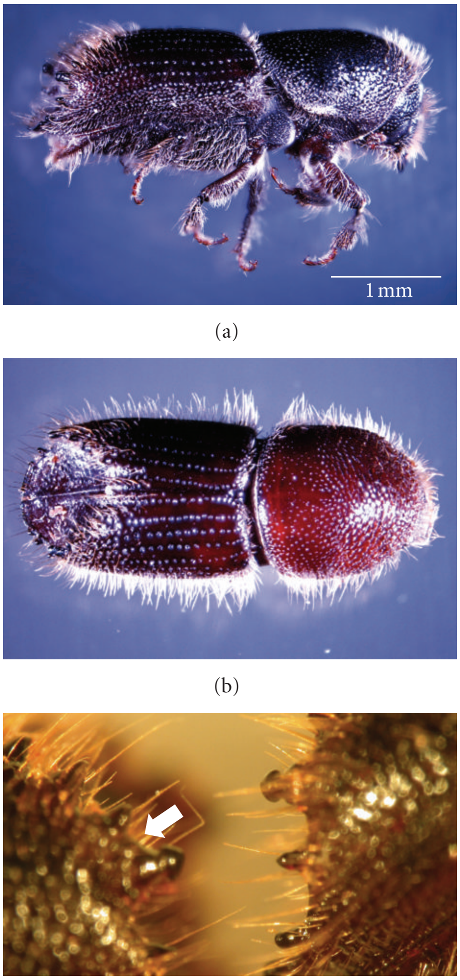
In January 2009, the windstorm "Klaus" struck the southern part of France, affecting 37.9 million m^3 of maritime pine *Pinus pinaster* Aiton (Pinales: Pinaceae). This breeding plant material favored the outbreak of *Ips sexdentatus* (Börner) (Coleoptera: Curculionidae: Scolytinae). As much of this timber is imported to the Basque Country (northern Spain), a potential risk to conifer stands is generated, due to the emergence of the incoming beetles. Thus, flight dynamics and beetle abundance were compared in different sawmills, according to the timber species (either local *P. radiata* D. Don or imported *P. pinaster*). A maximum flight peak of *I. sexdentatus* was observed in mid-June in *P. pinaster* importing sawmills, whereas a second lighter peak occurred in September. In contrast, only a maximum peak in mid-June was observed in *P. radiata* inhabiting beetles, being significantly smaller than in local *P. pinaster* trading sawmills. In addition, significant differences were found between imported *P. pinaster* and *P. radiata* regarding the number of insects beneath the bark. The development of IPM strategies for controlling *I. sexdentatus* populations is recommended, due to the insect abundance found in *P. pinaster* imported timber.

1. Introduction

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are an insect group that contains at least 6,000 species from 181 genera around the world [1]. Bark beetles are considered as important agents of forest succession and initiate the sequence of nutrient cycling in infested tree material [2]. However, it is well known that some species are among the most destructive insects of coniferous forests, representing a continuous threat [1, 3]. Although bark beetles tend to colonize dead or weakened trees, it is well reported that some species can attack healthy trees under epidemic conditions. Frequently, improper forestry management or adverse abiotic and climatic conditions (e.g., storms, fires, and droughts) act as precursors by providing breeding substrate that unleashes population outbreaks for these bark beetles species [4–6]. For instance, the storms "Vivian/Wiebke" in February/March 1990 and "Lothar" in December 1999 triggered the propagation of *Ips typographus* (L.) in Centre Europe [7]. Recently, "Klaus" named windstorm affected 37.9 million m^3 of maritime pine *Pinus pinaster* Aiton (Pinales: Pinaceae) in Aquitaine (southern France) during January 2009 [8]. As a consequence a great amount of windthrown timber was left as suitable breeding material for the six-toothed beetle *I. sexdentatus* (Börner) Figure 1. Despite its preference for weak, decaying or dead trees, the six-toothed beetle can attack healthy trees under outbreak conditions. Much of this timber from Landes region is imported to many sawmill and timber-processing industries located at the Basque Country (northern Spain), due to its low cost. The long-time storage of such infested logs could put into risk the local forestry management, since new emerging *I. sexdentatus* would disperse beyond sawmills and attack the adjacent Monterey pine (*P. radiata* D. Don) stands,



(a)

(b)

(c)

Figure 1: *Ips sexdentatus* (Börner) (Coleoptera: Curculionidae: Scolytinae), lateral (a) and dorsal views (b), and detail of the elytral declivity of male (left) and female (right). Note the fusion at the base of the 3rd and 4th teeth in male (white arrow).

which is the most common tree species planted in the Basque Country [9].

Ips sexdentatus is a Palearctic species distributed throughout Europe which is capable of breeding in many coniferous genera, including *Pinus* L., *Picea* A. Dietr. (Pinaceae), *Larix* Mill. (Pinaceae), and *Abies* Mill. (Pinaceae) [10, 11]. Concerning the Basque Country, it has been trapped in both *P. radiata* and *P. sylvestris* L. stands [12]. It is associated with several species of ophiostomatoid fungi (Sordariomycetes: Ophiostomatales) [13, 14], which are involved in many tree diseases and sapstain [15]. Not only with blue-staining fungi, but also the association with the fungus *Fusarium circinatum* Niremberg and O'Donnell (= *F. subglutinans* f. sp. *pini* Correll et al. (Hypocreales: Nectriaceae), causal agent of the pitch canker disease, has been detected in *P. radiata* inhabiting populations in the Basque Country [16].

Thus, the aim of the current work was to determine the flight dynamics of *I. sexdentatus* in different sawmills from the Basque Country, according to different timber species (either *P. radiata* or imported *P. pinaster*). Secondly, in order to evaluate the infestation level of maritime pine, the density of beetles was evaluated, through direct observation on debarked logs. These primary objectives would allow inferring the significance and risk of importing maritime pine to the Basque Country.

Table 1: Sampling sawmills located at the Basque Country (northern Spain). *Pinus* L. species (Pinales: Pinaceae) is also indicated within each row.

Locality	Province	Latitude and longitude	Timber
Amezketa	Guipuzcoa	43° 02′ N, 02° 04′ W	*P. pinaster* Aiton
Tolosa	Guipuzcoa	43° 07′ N, 02° 04′ W	*P. pinaster*
Aia	Guipuzcoa	43° 15′ N, 02° 09′ W	*P. pinaster*
Berrobi	Guipuzcoa	43° 08′ N, 02° 01′ W	*P. radiata* D. Don
Zalla	Biscay	43° 12′ N, 03° 08′ W	*P. radiata*
Legutiano	Alava	42° 58′ N, 02° 38′ W	*P. radiata*

2. Material and Methods

Monitoring trapping took place from 1st April to 31th October 2011. Six different commercial sawmills were chosen. Three of them use *P. radiata* planted in the Basque Country as primary resource, whereas the other three import maritime pine timber from Landes region (southwestern France). The locations of sampling sites are provided in Table 1.

Two eight-unit Lindgren multiple funnel traps (Econex S.L., Murcia, Spain) were placed in each sawmill. Each trap was hung with the top of the trap at 2 m above the ground and the distance between traps was at least 50 m. One trap was unbaited, as a blank control, whereas the other trap was baited with a synthetic *I. sexdentatus*-specific pheromone (a mixture of ipsdienol (212.9 mg), *cis*-verbenol (60.8 mg), and ipsenol (13.6 mg), SEDQ, Barcelona, Spain). Baits were replaced every two months. Fifty mL of propylene glycol were added to each trap cup to kill and preserve captured insects. Not only *I. sexdentatus*, but also other bark beetles species and other accidentally trapped beetles were collected. Samples were removed every fifteen days and taken to the laboratory. Voucher specimens have been deposited at the Entomology Collection of the NEIKER-Basque Institute for Agricultural Research and Development, Arkaute, Basque Country, Spain.

In order to determine what *Pinus* species showed the largest density of *I. sexdentatus*, sections of 70 cm × 30 cm of seven randomly chosen logs (from both *P. radiata* and *P. pinaster*) were peeled off every week from 2nd May to 31th July in each sawmill. Debarking was made with the aid of a chisel. All *I. sexdentatus* present in the galleries beneath the bark were collected. The number of galleries was also recorded.

Data of mean catches of flying beetles caught in baited traps were subjected to a two-way ANOVA analysis (with pine species and date considered as factors). Subsequent Tukey *post-hoc* tests at a significance level of $\alpha = 0.05$

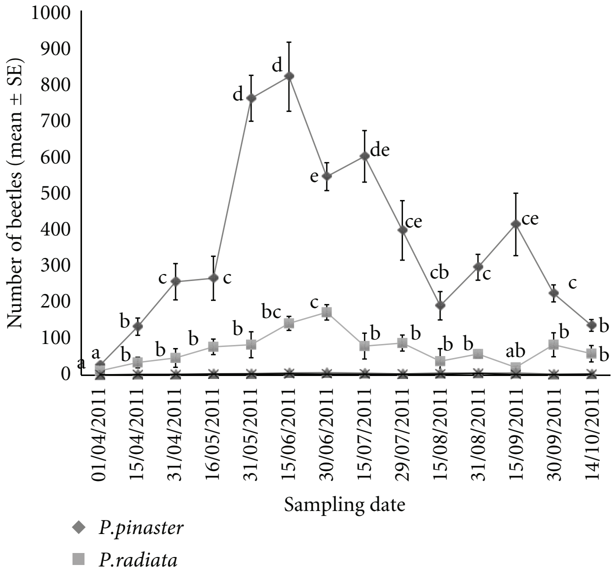


Figure 2: Number (mean ± SE) of *Ips sexdentatus* (Börner) (Coleoptera: Curculionidae: Scolytinae) captured in *Pinus pinaster* Aiton (Pinales: Pinaceae) (dark grey) and *P. radiata* D. Don (light grey) sawmills from 1 April to 14 October 2011. Dates within each *Pinus* L. species with different letters are significantly different at a significance level of $\alpha = 0.05$. Control catches in both cases were insignificant to perform any statistical analysis.

were applied to compare mean catches between dates within each *Pinus* species. Concerning density data, Student's t-test was used to compare mean number of galleries and beetle collections in different *Pinus* species for each month. A square root transformation was used to normalize the data and correct the heteroscedasticity. All the analyses were performed with the statistical software SPSS 2004 SYSTAT statistical package (version 13.0, SPSS, Chicago).

3. Results

A total of 15,184 specimens of *I. sexdentatus* were trapped in *P. pinaster* importing sawmills, whereas 2,774 were captured in *P. radiata* sawmills. As expected, pheromone-baited traps caught significant more insects in *P. pinaster* sawmills when compared with captures in *P. radiata* sawmills ($F = 108.927$, df = 1, $P < 0.001$). An interaction between sampling dates and *Pinus* species was found ($F = 7.2440$, df = 13, $P < 0.001$). A maximum flight peak was observed from the end of May to middle June for maritime pine, whereas a slighter peak occurred on September (Figure 2). Regarding *P. radiata* sawmills, a significant peak was observed only at the end of June. No statistical differences were observed in catches of other accidentally trapped insects.

Significant differences were found between the mean number of beetles and galleries under the bark during the three months. Maritime pine sections showed significant more galleries (May: $t = 4.152$, df = 12, $P = 0.002$; June: $t = 5.928$, df = 12, $P < 0.001$; July: $t = 5.063$, df = 12, $P < 0.001$) (Figure 3(a)) and beetles (May: $t = 9.367$, df = 12, $P < 0.001$; June: $t = 8.538$, df = 12, $P < 0.001$; July: $t = 7.900$, df = 12, $P < 0.001$) (Figure 3(b)) than in local *P. radiata*.

In addition, many other bark and ambrosia beetles species were accidentally captured in pheromone-baited traps. Table 2 details the different bark and ambrosia beetles caught per locality, along with other xylophagous species (Coleoptera: Cerambycidae) and bark beetle predators (Coleoptera: Cleridae).

4. Discussion

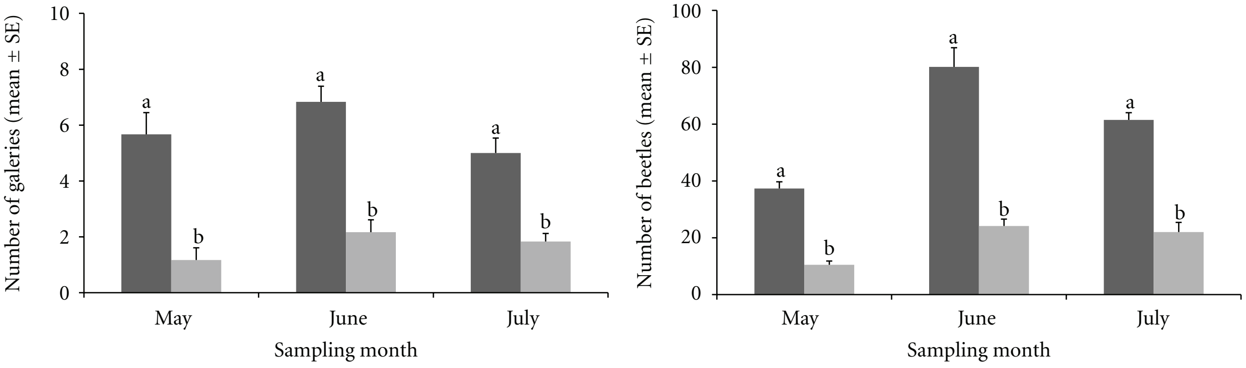
Current work demonstrates that maritime pine timber imported from France to commercial sawmills is highly infested compared to *P. radiata* timber, according to observed differences in the amount of insects caught in both field trapping and log debarking.

The six-toothed beetle has two generations per year, with adult flight periods from April to May and July to August. However, *I. sexdentatus* can undergo a third generation in Mediterranean regions of Europe [17]. Our results are consistent with other studies. Similar maximum flight peaks have been observed in *Picea orientalis* (L.) Link (in Turkey) and *Pinus sylvestris* (in Romania) stands [18, 19]. In contrast, *I. sexdentatus* showed three different peak flights in *P. pinaster* stands at the province of Leon (northern Spain), with the maximum peak occurring in September [20]. It has been suggested that this latter increase might be due to a strong increasing of the population during that season or a seasonal pheromone production, as it occurs in *I. pini* (Say) [21].

Ips sexdentatus is a polygamous species in which male is the pioneer sex which initiates the host seeking process. Afterwards, up to 2–5 females join each male within the gallery systems [10]. Galleries are star shaped, with a central nuptial chamber built by the male and in which mating occurs. Females bore egg galleries, which radiate outwards from the nuptial chamber. All the observed galleries in the current study had more than two arms.

Among accidentally trapped bark beetles species, it is worth noting the find of a female exemplar of the small spruce bark beetle *Polygraphus poligraphus* (L.), which would represent the first record for the Iberian Peninsula. *Polygraphus poligraphus* inhabits *Picea abies* (L.) H. Karst. and *P. obovata* Ledeb. [11], rarely breeding in *Pinus sylvestris* and *P. strobus* L. [10, 11, 22]. This unique specimen was trapped in the sawmill located at Berrobi, in which *P. radiata* timber is used. In addition, its distribution area is supposed to extend from Central Europe to Northern Europe and Siberia [11], being absent in the Mediterranean region [10]. Thus, the presence of this insect in the sampling area should be clearly stated.

Moreover, two species of *Monochamus* Dejean (Coleoptera: Cerambycidae) were also trapped, mainly in two *P. pinaster* trading sawmills: *M. sutor* (L.) and *M. galloprovincialis* (Olivier). The latter shows special relevance, as it is known to be the vector of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle (Aphelenchida, Parasitaphelenchidae), causal agent of the pine wilt



(a) (b)

Figure 3: Number (mean ± SE) of (a) galleries and (b) *Ips sexdentatus* (Börner) (Coleoptera: Curculionidae: Scolytinae) found under the bark of *Pinus pinaster* Aiton (Pinales: Pinaceae) (dark grey) and *Pinus radiata* D. Don (Pinales: Pinaceae) (light grey) logs from May to July ($n = 28$). Means within each month with different letters are significantly different at a significance level of $\alpha = 0.05$.

Table 2: Total number of accidentally trapped species of bark and ambrosia beetles (Curculionidae: Scolytinae), cerambycid (Cerambycidae) and checkered beetles (Cleridae). Species within family/subfamily are sorted by alphabetical order.

Species/Locality	Amezketa	Tolosa	Aia	Berrobi	Zalla	Legutiano
Coleoptera: Curculionidae: Scolytinae						
Dryocoetes autographus (Ratzeburg)	5	0	0	1	0	0
Dryocoetes villosus (F.)	9	0	3	2	0	0
Gnathotrichus materiarius (Fitch)	249	7	112	23	22	23
Hylastes ater (Paykull)	22	0	0	3	0	22
Hylurgops palliatus (Gyllenhal)	2	0	1	0	0	0
Hylurgus ligniperda (F.)	137	20	33	3	1	3
Kissophagus hederae (Schmitt)	3	0	0	0	0	0
Orthotomicus erosus (Wollaston)	111	14	57	2	26	95
Orthotomicus laricis (F.)	86	0	18	7	12	1
Pityogenes calcaratus (Eichhoff)	11	1	0	0	9	54
Polygraphus poligraphus (L.)*	0	0	0	1	0	0
Xyleborinus saxeseni (Ratzeburg)	0	0	0	0	0	16
Xyleborus eurygraphus (Ratzeburg)	35	0	11	0	0	0
Xyleborus dryographus (Ratzeburg)	1	0	7	1	0	0
Xylosandrus germanus (Blandford)	1	0	4	2	0	0
Coleoptera: Cerambycidae						
Monochamus galloprovincialis (Olivier)	12	0	2	0	0	0
Monochamus sutor (L.)	28	0	4	0	0	1
Coleoptera: Cleridae						
Allonyx quadrimaculatus (Schaller)	2	0	0	0	0	0
Clerus mutillarius F.	1	0	0	0	0	0
Thanasimus formicarius (L.)	337	157	33	43	25	45

*Indicates first record for the Iberian Peninsula.

disease in different countries, including in Europe (Portugal and Spain) [23–26]. The kairomonal attraction to bark beetle pheromone components has been previously reported in some long-horned beetles, including *M. galloprovincialis* in Spain, another North American species of the genus [27–29].

The checkered beetle *Thanasimus formicarius* (L.) (Coleoptera: Cleridae) was the most common predator found in traps (527 individuals in *P. pinaster* sawmills and 113 in *P. radiata* sawmills). This insect is a common predator of European conifer bark beetles [30], and it is capable of locating their preys by detecting bark beetle produced-pheromones as kairomonal signals [31]. Moreover, it has been reported that they recognize conifer volatiles and even volatiles from angiosperm trees that act as nonhost volatiles to conifer bark beetles [32]. *Allonyx quadrimaculatus* (Schaller) is also considered as a predatorof *Tomicus* *piniperda* L. [33], although there are not concrete studies about the mechanisms involved in prey detection.

As in other species of the genus, management programs should be focused on minimizing attacks on living trees, the sanitation of infested trees and the establishment of a trapping system [7]. The use of semiochemicals with antiaggregative effects should be considered as a useful management tool for trees protection. (1*S*, 4*S*)-(-)-Verbenone (4,6,6-trimethylbicyclo-[3.1.1]hept-3-en-2-one, hereafter (-)-verbenone), has been demonstrated to be capable of disrupting the pheromone-mediated attraction of *I. sexdentatus* [16, 34]. Romón et al. [16] detected a significant negative dose-dependent relationship between different (-)-verbenone release rates (0.01, 0.2, 1.8, and 3.1 mg/24 h) and catches of *I. sexdentatus* in a *P. radiata* stand. Etxebeste and Pajares [34] also found significant reduction in catches when testing (-)-verbenone at 2 and 40 mg/day in a mixed pine stand (ca. 40-year-old *P. pinaster* with younger ca. 30-year-old *P. sylvestris*). In addition, the spyroketal *trans*-7-methyl-1,6-dioxaspiro[4.5]decane (commonly known as *trans*-conophthorin) has also shown promising results. There are evidences of its electrophysiological detection by *I. sexdentatus* [35],and the antiaggregative effect is supported by field assays, although with some disparities. Despite Jactel et al. did not find any significant reduction in trap catches when testing *trans*-conophthorin at 5 mg/day [35], a 16-time lower release rate (i.e., 0.3 mg/day) is capable of reducing the response of *I. sexdentatus* to aggregation pheromone [34]. Moreover, *trans*-conophthorin seems to achieve stronger effects when combined either with (-)-verbenone or NHV alcohols [34, 35]. Thus, taken into account these results, we suggest the development of “push-pull” strategies [36], using pheromone-baited traps inside the park (to favor insect mass trapping) and blends of disruptant semiochemicals at the edges of close pine stands, in order to repel incoming beetles. Long-time buildup of logs should also be not recommended. Future field studies are needed to evaluate the impact of these incoming *I. sexdentatus* populations upon local conifer stands.

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