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FLORAL ECOLOGY OF A NIGHT-BLOOMING PLANT IN ITS DISAPPEARING HABITAT: A COMPREHENSIVE STUDY OF FLOWER VISITORS, POLLINATION, AND FLORAL SCENT IN *GUETTARDA SCABRA* (RUBIACEAE)

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BIOLOGY

by

Maria Cleopatra Pimienta Idrobo

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To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Maria Cleopatra Pimienta Idrobo, and entitled Floral Ecology of a Night-blooming Plant in its Disappearing Habitat: A Comprehensive Study of Flower Visitors, Pollination, and Floral Scent in *Guettarda scabra* (Rubiaceae), having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

Florence George

Javier Francisco Ortega

Diego Salazar-Amoretti

Jamie Theobald

Suzanne Koptur, Major Professor

Date of Defense: June 8, 2023

The dissertation of Maria Cleopatra Pimienta Idrobo is approved.

Dean Michael R. Heithaus
College of Arts, Sciences and Education

Andrés G. Gil
Vice President for Research and Economic Development
and Dean of the University Graduate School

Florida International University, 2023

DEDICATION

To the memory of my loving father.

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ABSTRACT OF THE DISSERTATION

FLORAL ECOLOGY OF A NIGHT-BLOOMING PLANT IN ITS DISAPPEARING HABITAT: A COMPREHENSIVE STUDY OF FLOWER VISITORS, POLLINATION, AND FLORAL SCENT IN *GUETTARDA SCABRA* (RUBIACEAE)

by

Maria Cleopatra Pimienta Idrobo

Florida International University, 2023

Miami, Florida

Professor Suzanne Koptur, Major Professor

Knowledge about the floral ecology of native plants is fundamental for effective conservation efforts, as many of these species are at risk from habitat destruction and fragmentation. In south Florida, the imperiled pine rockland habitat holds numerous native plants that depend on animals for their reproduction. Many of these plants have not been studied and their floral ecology remains unknown, particularly night-blooming species, whose study is especially challenging. *Guettarda scabra* (Rubiaceae), a native night-blooming species in south Florida's pine rocklands, relies on insects for pollination. Three primary questions guide this study: (1) Which arthropods visit *G. scabra* flowers during the day and night? (2) Which are the plant's effective pollinators? (3) What is the chemical profile of *G. scabra*'s floral scent, and what role do its components have in attracting pollinators? Through field observations and controlled experiments, I provide a detailed account of the arthropods visiting flowers of *G. scabra*, along with a thorough evaluation of their role as potential pollinators in this species. I also characterize the chemical profile of the flower scent and explain how the compounds present are likely fine-tuned to the attraction of the most effective pollinator found. My findings show *G. scabra* is visited by a wide range of arthropods, mostly Lepidoptera and Hymenoptera whose populations rely on floral rewards from this species during late summer, when flowering season has passed for most of the other local plants. My experiments revealed that *G. scabra* has a specialized pollination system where nocturnal hawkmoths are the only effective pollinators, while diurnal visitors behave as nectar and pollen thieves. Finally, I found that the chemical profile of the floral scent in *G. scabra* fits that of night-blooming plants pollinated by nocturnal hawkmoths. My work highlights the critical role that *G. scabra* plays as a nectar resource in the dwindling pine rockland habitat, provides baseline data on the local diversity and natural history of insect flower visitors, and establishes a baseline knowledge of the chemical ecology of this species. My findings yield needed information to support efforts to conserve pine rocklands and the plants and animals that inhabit them.

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PREFACE

Chapter II has been published in the peer-reviewed journal *Plants* and is open access, so it can be reproduced freely here for non-commercial purposes with the citations provided below. Chapter III was submitted to the peer-reviewed journal *Flora*. Both chapters have been formatted following the journal guidelines.

CHAPTER II

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CHAPTER I

INTRODUCTION

Knowledge of the floral ecology of native plants is critical to ensure the success of conservation efforts and their continued existence. Many native species face a range of threats, including habitat destruction and fragmentation, leading to declining plant population sizes and reduction of pollinator diversity and abundance (Aizen and Feinsinger, 1994). Most flowering plants depend almost completely on visitors for pollen transfer, and hence sexual reproduction (Ollerton et al., 2011). Although many vertebrates can be pollinators, the vast majority of pollen vectors are insects; without them, many species of flowering plants would likely go extinct (Proctor et al., 1996).

The dependence of plants on insect pollinators for their reproduction has led to the coevolution of mutualistic relationships, in which interactions have been fine-tuned at different levels over evolutionary time (Willmer, 2011). The nature of this fine-tuning includes the time at which the flowers are open and ready to be pollinated, and is affected by the identity of the effective pollinators (Willmer, 2011). Plants attracting similar groups of insects tend to converge in their floral traits including morphology, color, scent, and rewards, due to their fine-tuning to the senses of their visitors (Faegri and van der Pijl, 1979; Willmer, 2011). These somewhat predictable patterns are known as pollination syndromes.

While plants with flowers open during daylight hours can rely on contrasting colors and shapes to attract pollinators, the same cannot be said about night-blooming species. Even though some nocturnal pollinators exhibit complex adaptations in their visual systems to navigate in the dark (Kelber et al., 2002; Greiner et al., 2004; Warrant, 2008; Somanathan et al., 2009), many species of night-blooming plants depend mostly on strong floral scents to attract visitors (Raguso, 2004; Borges et al., 2016).

Despite the ecological importance of nocturnal interactions between plants and pollinators and the fact that they can be disrupted by anthropogenic changes to ecosystems, research on nocturnal pollination is underrepresented and most of these nocturnal systems remain unknown (Macgregor and Scott-Brown, 2020). This is understandable, as night study entails challenges not presented during the day. To fully understand what happens in plants with flowers open at night, we must study them at night, as well as during the day.

The pine rockland habitat is unique to south Florida and the Caribbean and is considered critically endangered because of the loss of its original extent, due largely to anthropogenic development over the last century (Koptur, 2006; FNAI, 2010). In the state of Florida, outside the Everglades National Park, only 2% of its original area remains as fragments (Snyder et al., 1990; Koptur, 2006, Peña and Koptur, 2021). Night-blooming plants might be especially susceptible to the effects of fragmentation. In general, plants with nocturnal anthesis guarantee their reproduction by coevolving with certain groups of pollinators and becoming dependent on them (Willmer, 2011). For the plant, this mutualism is at risk if the habitat is altered enough to no longer guarantee the presence of the most effective pollinator when the plant is in bloom.

In south Florida, the pine rockland habitat contains a diverse understory of plants including numerous native species (Natureserve, 2022), many of which have not been studied and whose floral ecology remains unknown. This applies particularly to night-blooming species, whose direct observation is challenging. It is imperative to understand the interactions essential to the survival of plants native to this imperiled ecosystem in order to best conserve them and their pine rockland home.

One of the main components of the understory of the pine rockland habitat is the rough-leaf velvetseed *Guettarda scabra* (L.)Vent. (Rubiaceae), a night-blooming plant whose unknown pollinator is presumed to be hawkmoths (Sphingidae). This assumption has been traditionally based on the set of traits that *G. scabra* flowers exhibit, the “sphingophilous” syndrome (white and long tubular corolla, nocturnal anthesis, and the emission of a strong scent). However, the identity of the pollen vectors of this species remains unknown. Furthermore, preliminary observations show that the nocturnal flowers remain open through the morning and are recurrently visited by insects during that time. This pattern of floral behavior raises questions regarding the role of this plant as a source of rewards for the local arthropod community and whether the diurnal visitors play any role in the plant’s pollination and successful reproduction.

To fill these gaps in our knowledge, I sought to investigate the interactions between rough-leaf velvetseed and the local arthropod fauna visiting its flowers, from the perspective of the plant. To this end, I begin my dissertation with an account of arthropod activity on the flowers of *G. scabra*. I then evaluate the effect of diurnal versus nocturnal floral visitors on the plant’s reproduction and identify the effective pollinators. Finally, I present a comprehensive study of the chemistry of floral scent in this species, and its role in attracting those pollinators.

In chapter II, I present a detailed investigation of the arthropods visiting flowers of *G. scabra* in two areas containing pine rockland fragments in south Florida. I made observations both during day and night and described the activity performed by visitors on the flowers, as well as the nature of the floral resource they used. Based on my observations, I identified potential pollinators for *G. scabra* and I demonstrated the importance of floral resources offered by this dominant native plant in sustaining the local arthropod fauna. Chapter II has been published in the peer-reviewed journal *Plants*.

In chapter III, I focus on evaluating the relative contribution of both diurnal and nocturnal Lepidoptera to *G. scabra* successful reproduction. By using pollinator exclusion experiments and direct observations, I isolated the effect of visitors at during the day and at night, documenting their visitation frequencies. In this chapter, I show that fruit production in *G. scabra* depends exclusively on nocturnal hawkmoths, despite most of its floral visitors being diurnal. This chapter has been submitted for publication to the peer-reviewed journal *Flora*.

In chapter IV, I evaluate whether the chemical profile of the floral fragrance of *G. scabra* is consistent with that of a hawkmoth-pollinated plant. To that end, I used dynamic headspace sampling and GC-MS analysis to determine the identity and ratio of the chemical components of the floral scent of *G. scabra*. I found that the fragrance of the flowers in this species is dominated by the presence of benzenoid and terpenoid compounds that are characteristically attractive to nocturnal hawkmoths. These findings further support what I found in the exclusion experiment described in the previous chapter.

I present this dissertation as a contribution to understanding the plant/animal interactions of native plants of the pine rocklands. The genus *Guettarda* occurs widely, and I compare my results to those found in other species that have been studied around the world. I hope that my work will emphasize the importance of not only conservation of pine rockland plants, but also the entire habitat and the animals that play an important role in reproduction and continued existence of *G. scabra*.

References

Aizen, M. A., Feinsinger, P. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology,* 75: 330–351.

Borges, R. M., Somanathan, H. and Kelber, A. 2016. Patterns and processes in nocturnal and crepuscular pollination services. *The Quarterly Review of Biology,* 91(4): 389–418

Faegri, K., and van der Pilj, L. 1979. The Principles of Pollination Ecology. Pergamon Press, Oxford, UK.

Florida Natural Areas Inventory (FNAI). 2010. Pine Rockland. In Guide to the Natural Communities of Florida, 69–72. Florida Natural Areas Inventory, Tallahassee, FL, USA.

Greiner, B., Ribi, W. A. and Warrant, E. J. 2004. Retinal and optical adaptations for nocturnal vision in the halictid bee *Megalopta genalis*. *Cell and Tissue Research,* 316, 377–390

Kelber, A., Balkenius, A. and Warrant, E. J. 2002. Scotopic colour vision in nocturnal hawkmoths. *Nature,* 419, 922–925

Koptur, S. 2006. The conservation of specialized and generalized pollination systems in subtropical ecosystems: a case study. *In* Waser, N. and Ollerton, J. (eds.) Plant–pollinator interactions: from specialization to generalization. 341–361. University of Chicago Press. Chicago. Illinois, USA.

Macgregor, C. J. and Scott-Brown, A. S. 2020. Nocturnal pollination: an overlooked ecosystem service vulnerable to environmental change. *Emerging Topics in Life Sciences,* 4:19-32.

Natureserve. 2022. Available online: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.723149/South_Florida_Pine_Rockland (accessed on 19 October 2022).

Ollerton, J., Winfree, R., and Tarrant, S. 2011. How many flowering plants are pollinated by animals? *Oikos*, 120: 321–326.

Peña, A. L. and Koptur, S. 2021. A historical floristic inventory of pine rockland Fabaceae (Leguminosae). *Natural Areas Journal*, 41(4):258–272.

Proctor, M., Yeo, P. and Lack, A. 1996. The natural history of pollination. Harper Collins Publishers, London

Raguso, R. A. 2004. “Why do flowers smell? The chemical ecology of fragrance driven pollination.” *In* Cardé, R. T. and Millar, J. G. (eds.) Advances in insect chemical ecology. 151–178. Cambridge University Press. Cambridge, UK),

Snyder, J. R., Herndon, A., and Robertson, Jr., W. B. 1990. South Florida rockland. *In* Myers, R. L., and Ewel, J. J. (eds.), Ecosystems of Florida, 230–274. University of Central Florida Press, Orlando, FL, USA.

Somanathan, H., Kelber, A., Borges, R. M., Wallén, R. and Warrant, E. J. 2009. Visual ecology of Indian carpenter bees II: adaptations of eyes and ocelli to nocturnal and diurnal lifestyles. *Journal of Comparative Physiology A*, 195: 571–583

Warrant, E. J. 2008. Seeing in the dark: vision and visual behaviour in nocturnal bees and wasps. *Journal of Experimental Biology*, 211(11): 1737–1746.

Willmer, P. 2011. Pollination and floral ecology. Princeton University Press, Princeton, New Jersey, USA.

CHAPTER II

MORE THAN MOTHS: FLOWER VISITORS OF A NIGHT-BLOOMING PLANT IN SOUTH FLORIDA PINE ROCKLANDS, USA

María Cleopatra Pimienta and Suzanne Koptur

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Abstract

Plants whose flowers open at night but remain open during the day also attract diurnal flower visitors, potentially boosting their pollination rates and providing resources that can support diverse arthropod communities. The rough-leaf velvetseed, *Guettarda scabra* (Rubiaceae), is an evergreen shrub that thrives only in the imperiled pine rockland habitat in south Florida. Its white, tubular, and fragrant flowers open during late afternoon, exhibiting traits strongly associated with the attraction of nocturnal hawkmoths (Sphingidae). Flowers of *G. scabra* remain open until the following morning, becoming available to a wider array of visitors, bringing into question the expectation that sphingophilous flowers are visited mainly by hawkmoths. To evaluate whether the flowers of *G. scabra* are mainly visited by nocturnal hawkmoths and understand the role of this plant in the pine rockland habitat, we characterized the arthropod fauna associated with its flowers during the morning, evening, and at night. We found that most flower visitors were diurnal insects of the orders Hymenoptera and Lepidoptera, although we observed other arthropod groups too. Visitation at night was dominated by two species of hawkmoths. Nectar was the main resource used by the arthropod community during this study. Legitimate visitation and nectar-robbing were the behaviors most frequently observed among the flower visitors. Our results suggest that flowers of the night-blooming *G. scabra* constitute an important food source for both diurnal and nocturnal arthropod fauna in the fire-dependent pine rocklands of southern Florida. Our study provides novel data to support efforts to conserve and protect pine rocklands and the plants and animals that inhabit them.

Keywords

butterflies; floral resources; *Guettarda scabra*; hawkmoths; insects; nectar robbing; pine rockland; pollination

1. Introduction

Although floral morphology often suggests coevolution with determined pollen vectors, flowers usually attract other visitors too [1–3]. The availability of these visitors and the reproductive success of the plant are affected by the time at which flowers open and for how long they remain available for visits [4]. As such, night-blooming plants whose flowers remain open during the day are likely to receive diurnal visitations, boosting their pollination opportunities.

The rough-leaf velvetseed (Figure 1a), *Guettarda scabra* (L.) Vent. (Rubiaceae), is a tropical evergreen shrub native to the Caribbean, ranging from the northern parts of Colombia and Venezuela to the southern portion of Florida (USA) [5–8]. In south Florida, *G. scabra* grows only in the last remnants of pine rockland (Figure 1b) and hardwood hammock habitats on the peninsular mainland, where it is abundant [9,10]. Pineland *G.scabra* plants are short in stature and allocate much more energy to flowering and fruiting than do the tall individuals persisting in hardwood hammocks [6].

Guettarda scabra flowers exhibit a set of traits associated with the attraction of nocturnal lepidopterans, particularly hawkmoths (Sphingidae). Sphingophilous flowers are pale, with long-tubed corollas, and emit a strong sweet scent [11]. Anthesis in *G. scabra* happens during late afternoon [12], which led to the assumption that they were exclusively for night-time visitors [13], particularly hawkmoths [10]. Recent observations have shown that these flowers remain open through the following morning and are visited by butterflies [14], suggesting that they can be attractive to other visitors too, providing resources to a larger arthropod community. Despite its local abundance, and its presence in the disappearing pine rocklands, the structure of the community of flower visitors associated with *G. scabra* has not been studied in detail, even though *G. scabra* thrives in an imperiled habitat and allegedly depends upon pollinators whose populations may be declining [15].

To test the hypothesis that flowers of this species are mainly visited by nocturnal Lepidoptera, we observed flowering plants during day and night. Besides nocturnal lepidopterans, we expected to find many other visitors to the flowers, not only at night, but evening and morning, during times the flowers are open, but hawkmoths are not present. We thoroughly characterize the local arthropod fauna associated with flowers of *G. scabra*, their behavior, and floral resources they use. We offer insights into the role played by this native plant species in its rockland habitat and identify many *G. scabra* potential pollinators, providing the basis for a deeper understanding of its pollination biology and its role in supporting the arthropod community of this imperiled ecosystem. By learning more about the relationships *G. scabra* has with pine rockland fauna, we test the traditional view of pollination syndromes and also elucidate the multitude of interactions a single plant species may have. In this approach, our study may reach beyond its local rare habitat and be relevant to other plant species worldwide.

2. Results

Flowers of G. scabra were visited by 46 species of arthropods, belonging to 8 orders and 20 families (Table 1). Most visitors were insects from the orders Lepidoptera and

Hymenoptera (27 species total, vs. 17 other species; Fisher's exact test $p < 0.01$), making up 63% of all species recorded. The proportions of visitors in these two orders did not differ significantly (Fisher's exact test $p > 0.05$). The remaining were arachnids of the order Araneae, or insects belonging to the orders Coleoptera, Diptera, Hemiptera, Mantodea, and Blattodea (Figure 2).

2.1. Occurrence

Most species were found only in one of the study sites: 61% of the total number of species observed at Larry and Penny Thompson Park were unique to that site; 24% of species observed at Everglades National Park were observed only there. Only a small fraction of the total species observed (15%) was common to both sites (Table 1). The proportion of unique species observed at each site (80% at LPT, 61% at ENP) did not, however, differ significantly with Fisher's Exact Test. Most arthropods registered (76%) were seen exclusively during daytime (especially the morning hours), substantially more (Fisher's Exact test $p < 0.01$) than those observed to visit only at night (15.2%). An even smaller proportion (8.7%) of the species visited flowers both day and night (Figure 3).

Overall, visitors were observed 3x more frequently in the daytime observations than in the evening observation periods, and 6x more frequently than during the night. Lepidoptera were the order most commonly observed during the morning and night; Hymenoptera most commonly in the morning and more than twice as often as Lepidoptera in the afternoon. Araneae, Diptera, and Hemiptera much more common in morning and evening; Coleoptera most often observed at night.

Flowers of *G. scabra* were visited in the morning mainly by two butterfly species, *Heliconius charithonia* and *Agraulis vanillae*, and three species of skippers, *Asbolis capucinus*, *Polites baracoa*, and *Ephyriades brunnea* (Figure 3). Evening visits were dominated by *Pseudomyrmex gracilis* ants and crab spiders of the genus *Mecaphesa*; while at night the hawkmoths *Xylophanes tersa* and *Eumorpha fasciatus* showed the highest occurrence (Figure 3).

2.2. Visitor Behavior

We identified four behaviors among arthropods visiting *G. scabra* flowers: (a) legitimate visitation, consumption of pollen or nectar through the opening of the corolla tube involving contact with the anthers, stigma, or both and potentially resulting in pollination; (b) nectar robbing, consumption of nectar through a perforation of the corolla either made by the visitor itself (primary robber) or left by a previous visitor (secondary robber); (c) predation on other arthropods; and (d) herbivory, feeding on leaves or flowers (Table 1, Figures 4–6).

Overall, legitimate visitation and nectar robbing were the most common behaviors observed among the flower-visiting species (Figure 7). Fisher’s exact test showed those behaviors combined were substantially greater than the others combined ($p < 0.05$), but neither was significantly different from the other, nor were predation and herbivory different from one another. The same patterns were seen at both sites separately. More than half (56%) of flower visitors at ENP and 34% at LPT visited flowers legitimately, and these were mainly lepidopterans (Table 1, Figure 4). Nectar robbing was performed by 32% of the visitors observed at LPT, and by 39% at ENP, mostly Hymenoptera (Table 1, Figure

5). Of the nectar robbers, 75% acted as secondary nectar robbers (Table 1). Herbivory was performed by different groups of insects at both study sites (Table 1, Figure 6), while predation was only witnessed at LPT and performed by spiders and mantises (Table 1, Figure 6).

2.3. Resources Consumed by Visitors

Visitors obtained five types of resources from *G. scabra* plants: nectar, pollen, floral tissue, leaves, and small insects attracted to the plant serving as prey (Table 1). Nectar was by far the main resource consumed by the arthropod community overall (Fisher's exact test, $p < 0.01$) as well as in both ENP ($p < 0.01$) and LPT ($p < 0.01$) (Figure 8), mostly Lepidoptera and Hymenoptera (Table 1). A surprising result was that some insects consumed postfloral nectar secreted after the corollas fell, the first time this has been observed in *G. scabra*. Consumption of other resources involved 43% of visitor species at LPT and only 17% of them at ENP (Figure 8). Just as with predation, we did not witness any visitors feeding on floral tissue at ENP.

2.4. Taxonomic Diversity and Behaviors of Visitors

2.4.1. Lepidoptera

This order contains 15 of the 46 species found visiting flowers of *G. scabra* in both study sites, making it one of two orders of arthropods with the greatest species richness attracted to these flowers. Among Lepidoptera, 11 were skippers (Hesperiidae), butterflies (Nymphalidae, Papilionidae), and moths (Erebidae), while the remaining were hawkmoths (Sphingidae) (Table 1). Among the 15 species of Lepidoptera found, 5 were observed in both study sites (e.g., Figure 4a–c), 7 were seen only in LPT (e.g., Figure 4d–e), and 3 only in ENP (Figure 4f–h) (Table 1).

While most Lepidoptera visited flowers during the day, the hawkmoths (*Eumorpha fasciatus*, *Perigonia lusca*, and *Xylophanes tersa*) were observed exclusively at night (Figure 3). In general, hawkmoths approached the plants by flying fast through the vegetation and fed only on fresh flowers by hovering above the corolla with their proboscis extended. Moths tended to visit a couple of flowers per plant and then fly away, maintaining a low number of visits per night. Individuals of *E. fasciatus* were often seen hanging motionless on branches of different plants around 2130 h, after visiting flowers (Figure 4h).

In contrast to hawkmoths, butterflies and skippers were observed foraging more frequently and visiting most of the flowers available in a single plant before moving to a nearby individual. Their intensive foraging strategy often resulted in multiple individuals and species feeding simultaneously on a single plant, occasionally even on withered flowers. Butterflies and skippers fed by landing on flowers and inserting their proboscis, and at times part of their head, into the corolla tube to reach the nectar (Figure 4a–f), sometimes resulting in large amounts of pollen being deposited on their mouthparts.

Besides adult lepidopterans, caterpillars of the erebid moths *Calidota laqueata*, *Hypercompe scribonia*, and *Seirarctia echo* were found feeding on leaves of *G. scabra* (Figure 6e–g). None of the adults of these species were observed visiting flowers.

2.4.2. Hymenoptera

Hymenoptera was the other order with many species visiting flowers of *G. scabra* (30.4% of all recorded visitors). Over half of them (57%) were found exclusively at LPT and only one species (*Zethus slossonae*) observed in both study sites (Table 1). Most Hymenoptera observed were either wasps or bees, while ants were represented by only a few species (Table 1). The ants *Pseudomyrmex gracilis* (Figure 5e) and *Camponotus floridanus* (Figure 5f) were the most frequently found throughout this study (Figure 3), although only at LPT. Notably, both the carpenter bee *Xylocopa micans* (Figure 5c) and the honeybee *Apis mellifera* (Figure 5d) were a common sight in ENP, in contrast to the remaining species.

Hymenopterans were observed to be active exclusively during the day, except for *C. floridanus* which foraged during the night as well (Figure 3). All hymenopterans visiting flowers of *G. scabra* fed on nectar, except for *Dielis trifasciata* (Figure 4i, Table 1) that consumed only pollen by inserting its head into the corolla opening. *Euglossa dilemma* was the only hymenopteran feeding on nectar through the natural opening of the flower while hovering over it, whereas *X. micans*, *Z. slossonae*, and an unidentified vespid wasp (Vespidae sp. 1) actively pierced the base of the corolla to access nectar (Figure 5a,c). Notably, individuals of *X. micans* observed during this study landed on the flowers and positioned themselves facing the base of the corolla, with their abdomen directed toward the flower opening. As the large bee cut the corolla tube, its abdominal hairs were rubbed against the anthers and sometimes the stigma (Figure 5c).

Remaining Hymenoptera acted as secondary nectar robbers, except for the wasp *Stenodynerus* sp. (Figure 5b), the only species behaving as both primary and secondary nectar thief. Ants moved through the plants constantly, exploring flowers to feed on nectar even after corolla abscission, upon which they visited post-floral nectaries (Figure 5i). Whenever scale insects or aphids were present, ants were seen protecting them and feeding on honeydew, which led to some aggressive interactions observed in LPT between *Wasmannia auropunctata* and *P. gracilis*.

2.4.3. Coleoptera

Flowers of *G. scabra* at ENP were visited at night by two species of long-horned beetles (Cerambycidae) that fed on pollen: *Eburia stigma* (Figure 4j) and *Plectromerus dentipes*. These beetles flew through the vegetation visiting one or two flowers per plant, where they were seen feeding on secretions of the stigma and inserting their head into the corolla to reach the pollen on the anthers (Figure 4j). In contrast, plants at LPT were visited by two species of scarab beetles (Scarabaeidae) (Table 1). *Euphoria sepulcralis* (Figure 4k) fed on pollen during the morning (Figure 4k), while *Phyllophaga* sp. was observed consuming open flowers and large buds at night, after 2100 h (Figure 6i).

2.4.4. Other Insect Orders

Observations of flies visiting flowers of *G. scabra* were uncommon (Table 1). There was a single morning sighting of the flower fly *Ornidia obesa* at LPT, during which the fly hovered before landing on flowers to consume pollen through the natural opening of the corolla, contacting the exposed stigma with its mouthparts in the process. Additionally, two species of crane flies (Tipulidae) were found acting as secondary nectar robbers during the day, feeding through holes available at the base of the corolla at both study sites.

The only Hemiptera observed at both study sites were *Largus succinctus*, a secondary nectar robber (Figure 5g). Occasional observations of aphids (Aphididae) extracting sap from flowers and buds only occurred at LPT (Figure 6h). Aphids were often accompanied by *C. floridanus* ants (Figure 5j), and in one instance also by a silver fly *Leucopis* sp. (see [16]). Scale insects (Coccoidea) were also found on inflorescences at LPT, but their presence was not recorded systematically.

Finally, two groups of Orthoptera were found only at LPT: a species of cockroach (Blattodea) acting as a secondary nectar robber and also visiting post-floral nectaries at night (Figure 5h); and two species of praying mantises (Mantidae) perched at the base of the inflorescences during daytime (Figure 6d).

2.4.5. Aranae

Five species of spiders were observed during this study, all of them at LPT: the orbweaver spider *Acacesia hamata* and four crab spiders of the genus *Mecaphesa* (Table 1). All spiders were observed sitting on the corolla, close to the pistil in both fresh and withered flowers, as well as on inflorescences with unopened buds (Figure 6a–c). They were observed either capturing small insects or resting on a flower with their front legs held out to each side of their body, a characteristic pose in this group.

3. Discussion

Although it was previously assumed that *G. scabra* is a moth-pollinated plant, our findings show that their flowers are visited by a wide array of arthropods that can act as pollinators, most of them diurnal. Such diversity is not surprising, since nearly 30% of arthropods species visit flowers regularly and potentially pollinate them [17]. Likewise, differences in diversity of visitors between night and day occur in many other plants whose flowers exhibit sphingophily, particularly the diurnal dominance of Hymenoptera and Lepidoptera that we observed in *G. scabra* (e.g., [18–21]), both groups being the largest insect taxa containing important pollinators [17].

In general, night-blooming species whose flowers remain open into the morning may be attractive to diurnal visitors, especially those unvisited flowers that accumulated nectar through the night [22]. Diurnal visitation of nocturnal flowers by a variety of animals has been reported across different families of plants. Examples highlighting the taxonomic diversity of plants include species of the families Caprifoliaceae and Cactaceae that are visited by bees [19,23], Apocynaceae and Rubiaceae by bees and butterflies [18,24], and Bromeliaceae by bees, ants, and flies [25]. The availability of nectar in the morning can even attract hummingbirds, as observed in Bromeliaceae [25] and Rubiaceae [26]. In the latter family, the genus *Guettarda* contains several species with this pattern of anthesis in which sphingophylous flowers remain open through the morning making nectar and pollen available to diurnal visitors. Observations on *G. speciosa* in south China [27] and *G. clarensis* in Cuba [28] revealed that both species were visited by a single local species of nocturnal hawkmoth and some diurnal insects, mostly lepidopterans, hymenopterans, and dipterans. While these two species were visited by both nocturnal and diurnal insects, *G.*

platypoda in Brazil was solely visited by nocturnal moths of three species [29]. These observations contrast with our findings, since *G. scabra* flowers are visited by a much larger number of species during the day and night. However, the frequency of visits by nocturnal hawkmoths was as low as in *G. platypoda* [29] and *G. speciosa* [27] (M.C.P. unpublished observations). Attracting a larger set of flower visitors may be advantageous for *G. scabra,* as non-hawkmoth visitors may provide some pollination when specialized hawkmoth pollinators are not available.

3.1. Occurrence

Our findings suggest that the flowers of *G. scabra* are visited by a community of arthropods whose structure differs between study sites. These differences may be linked to variations in the availability of biotic components of the ecosystem that depend on the presence of particular species of arthropods. Some of the species visiting flowers *of G. scabra* may require other resources that can vary between study sites, such as the presence of host plant species in the case of Lepidoptera, or nesting and shelter spaces for other arthropods. Carpenter bees (*X. micans*), for example, rely on the availability of dead wood they need to build their nests [30]. The scarcity of this resource might explain the absence of this species in LPT. On the other hand, our observations of the skipper *E. brunnea* in both study sites are clearly related to the availability of its host plant *Byrsonima lucida* (Malpighiaceae) [31] in both areas.

Surprisingly, the lepidopterans *Heliconius charithonia*, *Polites baracoa*, *Cymaenes tripunctus*, and *Papilio polyxenes,* which were all reported present all year round in the Long Pine Key area of ENP more than 40 years ago [31], were not observed in that area during this study, although we did observe them visiting flowers in LPT. Other notable absences in ENP include the caterpillars of three erebid moths (*Seirarctia echo, Spilosoma virginica*, and *Pyrrharctia isabella*), a paper wasp (*Mischocyttarus* sp.) and a species of flower fly (*Copestylum mexicanum*) seen visiting flowers of *G. scabra* over 30 years ago [14]. However, *S. echo* and a species of *Mischocyttarus* were found on *G. scabra* in LPT.

Interestingly, almost 25% of the total number of arthropod species found visiting flowers of *G. scabra* were only at ENP, a site that we undersampled with respect to LPT. While the sampling effort was different enough between both sites to prevent us from drawing any solid conclusions, the high proportion of species found only in ENP suggests that the communities of floral visitors are indeed different between study sites. It is possible that the arthropod community associated with *G. scabra* flowers in south Florida is even more taxonomically diverse than reported here.

3.2. Potential Pollinators

Guettarda scabra is visited by a wide range of potential pollinators besides lepidopterans. In fact, plants whose flowers fit a particular pollination syndrome may still receive visits from opportunistic insects capable of contributing to their fitness [3,23,32].

Due to floral morphology in *G. scabra*, most of their visitors with short mouthparts (such as bees, wasps, flies, and beetles) encounter anthers, stigma, or both while foraging, potentially serving as pollen vectors for this plant. Since anthers in flowers (of all morphs) of *G. scabra* are located at the opening of the corolla, short-tongued visitors can access pollen grains in any open flower and may then transfer them to flowers with exserted stigmas. Successful pollination of flowers with long corollas by short-tonged insects has been observed in other distylous Rubiaceae, such as *Psychotria homalosperma*. While that plant is presumably pollinated by long-tongued moths, in their absence, honeybees (*Apis mellifera*) manage to pollinate it with their short mouthparts by moving pollen unidirectionally from short- to long-styled flowers [33]. We think that a similar scenario occurs in *G. scabra*, where both short- and long-tongued visitors may promote pollination.

While short-tongued visitors could contribute to reproduction in *G. scabra*, floral traits in this species suggest the existence of a most effective pollinator with long mouthparts capable of encountering the stigma, regardless of how deep in the corolla it is located. Regarding the identity of such pollinator, previous authors have found nocturnal hawkmoths to be the main pollen vectors for other species in the genus *Guettarda*, such as *G. platypoda* [24,29], *G. speciosa* [27], and *G. clarensis* [28]. In fact, the nocturnal hawkmoth *X. tersa* was a common flower visitor of *G. scabra* during this study, and the same hawkmoth was the most frequent pollinator for *G. platypoda* in Brazil [29], suggesting a particular association between this moth and *Guettarda* plants when both are present. Besides lepidopterans, the bee *E. dilemma* was the only other visitor with a tongue long enough to reach nectar deep in the corolla of the flowers of *G. scabra*. This bee has a mutualistic relationship with orchids in its Central American native range, and was recently introduced to south Florida, where it has been reported (as *E. viridissima*) visiting a wide variety of non-orchid plants [34]; (Brittany M. Harris, personal communication). Our study provides the first record of *E. dilemma* visiting flowers of *G. scabra*.

Attracting different types of potential pollinators could enhance fruit production in areas where the most effective pollinator is absent or scarce [19], and/or when weather conditions disrupt foraging activity [35]. This plant has survived in the highly fragmented pine rockland habitat, being regularly exposed to extreme weather events such as heavy rainfall and flooding, hurricanes, and fire. Despite the low frequency of visits by nocturnal hawkmoths locally (M.C.P. unpublished observations), flowers of *G. scabra* may increase their chances of being pollinated by receiving visits from other pollen vectors observed during this study. In fact, day-active flower visitors may complement the effect of nocturnal ones in this species, as has been suggested by indirect observations [36].

3.3. Nectar Robbing: A Common Behavior

The fact that nectar robbing was a very common behavior observed among the floral visitors of *G. scabra* agrees with other instances in which more than half of the species of flower visitors are nectar robbers [37]. This behavior is known to happen in other species of the genus *Guettarda*. In *G. clarensis*, for example, nectar robbing reduces fruit production, negatively impacting reproduction [38]. Interestingly, the main robbers in *G. clarensis* (*Largus sellatus* and *Xylocopa cubaecola*) belong to the same genera as two common robbers we found in *G. scabra* (*L. succinctus* and *X. micans*) [39]. Nectar robbing has also been reported in *G. speciosa* [27], but there is no detailed account of these observations. In *G. scabra*, we did not observe damage caused by nectar robbers on sexual structures of the flowers (i.e., pistil or stamens), which could directly interfere with pollination, but it is unknown whether robbing can affect reproduction in this species.

While nectar robbing may be detrimental for plant reproduction [40], under certain conditions it may also have positive effects [41], such as contributing to pollination. Some *Xylocopa* bees for example, have been reported robbing nectar from plants with long tubular flowers [33,42–46]. In certain cases, they have been seen touching the anthers and stigma of flowers as they feed, promoting pollination [44–47]. Our observations on the foraging behavior of *X. micans* suggest that these bees may transfer pollen in *G. scabra* during nectar robbing. However, no other nectar robber observed during this study behaved or positioned its body in a way that could result in pollen transfer while they were feeding.

In addition to robbers depositing pollen, they may benefit the plants they rob in another way: by causing floral visitors to visit fewer flowers and move to other plants more quickly [48]. This is especially beneficial in plants that are self-incompatible [49] but may be important in avoiding inbreeding depression in those that are self-compatible as well by reducing geitonogamy [50]. As *G. scabra* is self-compatible, it may benefit from the actions of its numerous nectar robbers.

3.4. Guettarda Scabra as Food Source for Local Arthropod Fauna

Floral resources can be a limiting factor in many habitats during a particular season. In pine rocklands, most species flower from January to April [51] and initiate fruit formation during summer [52], reducing the availability of floral resources during this time. In contrast, most *G. scabra* individuals are fully in flower in June and July, when few other species are flowering, making them a valuable source of floral rewards. Our findings suggest that *G. scabra* may be a keystone species in the pine rockland habitat of south Florida, as it is an important source of food and foraging grounds for the local arthropod fauna during its flowering season. Flowers of this plant provide highly nutritious resources in the form of pollen and nectar to visitors, as well as flower parts and leaves for herbivores, making this plant attractive to a large variety of arthropods with diverse natural histories.

In fact, *G. scabra* flower rewards are used by wasps found only in Florida, such as *Z. slossonae* [53] and *D. trifasciata* [54]. Flowers of *G. scabra* also provide nectar for adult lepidopterans with distributions restricted to the southern half of Florida, such as *Perigonia lusca* [55] and *Cymaenes tripunctus* [56], along with *Ephyriades brunnea* whose populations have declined in recent years [57]. Such a critical role in the maintenance of the local pollinator fauna was observed also in *G. platypoda* in Brazil, where hawkmoth communities rely on its nectar as an energy source [24]. Although most adult lepidopterans visit *G. scabra* to feed on nectar, *H. charithonia* probably also consumes pollen, a resource reported as part of its diet [58–60]. Besides the erebid moths reported in this study, *G. scabr*a is the host plant for caterpillars of other species of moths in south Florida, such as *Spilosoma virginica* and *Pyrrharctia isabella* [14], as well as the hawkmoths *P. lusca* and *Eupyrrhoglossum sagra* [55,61].

Our observations also suggest that the pollen of *G. scabra* is an important food source for local populations of some long-horned and scarabeid beetles. In fact, scarabeids may rely on more than pollen from this plant, since at least *Phyllophaga* sp. was observed consuming its flowers during this study. It is also possible that *Euphoria sepulcralis* feeds on flower tissue of *G. scabra* as well, based on field observations of this species consuming flowers of other plants in LPT, including *Bidens* sp. (Asteraceae), *Spermacoce* sp. (Rubiaceae), and *Lantana* sp. (Verbenaceae) (M.C.P. personal observations), and occasional reports of this species as flower-damaging pest in some fruit trees in south Florida [62].

Besides insects, spiders may spend time on flowers benefiting from food sources other than prey. Spiders can feed on stigma exudates, nectar, and pollen [63–68]. While we did not witness this behavior directly, we often saw individual spiders sitting on the corolla, with their mouthparts very close to the stigma, anthers, or postfloral nectaries. Considering that the stigma of *G. scabra* remains moist throughout anthesis, and even after the corolla tube is wilted, spiders may have been feeding on stigmatic exudates. Interestingly, most of the spiders observed on flowers of *G. scabra* belong to the family Thomisidae, a group also commonly observed on flowers of G. *clarensis* [28].

The effect of predatory visitors on the reproductive success of *G. scabra* is unknown. In general, predators can harmfully disrupt pollination by consuming pollen vectors [69,70] or decreasing the frequency and duration of their visits [71–75]. Sometimes predators may benefit plants by causing pollinators to move between plants more [76], promoting outcrossing [77] as can nectar robbers [48]. At the same time, they can benefit the plant by decimating insects feeding on it [78]. In fact, some of the wasps observed during this study are known to attack phytophagous larvae, such as *Pachodynerus erynnis* that feeds on caterpillars of several families [79,80], or *D. trifasciata* which parasitizes larvae of the beetle *Phyllophaga portoricensis* [81]. Interestingly, we found a species of *Phyllophaga* consuming flowers of *G. scabra*, raising the question of whether *D. trifasciata* can control the local population of this beetle and benefit *G. scabra* in the process.

4. Materials and Methods

4.1. Plant Species

The rough-leaf velvetseed *Guettarda scabra* (Rubiaceae) is a tropical shrub usually less than 1.5 m tall when it grows in pine rockland forests in south Florida. Its blooming season begins in April and peaks between May and July [12]. Plants resprout after fire, but do not bloom the summer after burning, taking two years from fire until blooming again [36]. Flowers are white, often with a pink-flushed corolla tube, about 2 cm long that holds nectar at its base (Figure 1a). Flowers are arranged in dichasial cymose inflorescences and open sequentially over several weeks, usually one to three flowers per inflorescence per day, releasing a strong, sweet scent. Anthesis occurs during late afternoon and flowers remain fresh through the following morning [10]. Flower senescence occurs usually by noon, when the corolla turns brown and dehydrates, remaining attached to the calyx for about a day [10].

Guettarda scabra exhibits a special case of distyly, in which both the anther height and style length vary continuously in the population [10]. Plants are self-compatible, sometimes setting fruit without visitation, but pollen vectors are required for greater fruit production [10].

4.2. Study Sites

This study was conducted in two natural areas in Miami-Dade County, Florida, USA: (a) Larry and Penny Thompson Memorial Park (LPT), a county park containing the largest fragment of pine rockland habitat in the city of Miami (25°35′55″ N 80°23′55″ W); and (b) the Long Pine Key area (25°24′13.2″ N 80°39′33.2″ W), within a large, continuous pine rockland forest in Everglades National Park (ENP) (Figure 1b). The pine rockland habitat is unique to south Florida and the Caribbean and is considered critically imperiled due to a substantial loss of its original extent [82,83]. Although the objective of this study was not to compare the two sampling sites, for some aspects the data are shown separately to discuss general trends.

Rockland habitats are greatly reduced from their original extent as they have undergone extensive human development over the last century [83–85]. Pine rocklands are considered globally imperiled [86] with many endemic plant taxa in the diverse understory of more than 225 native plant species, of which 10% are considered threatened or endangered at the state level, eight of which are federally endangered [87].

4.3. Flower Visitor Observations

We surveyed arthropods visiting *G. scabra* flowers and/or feeding on the plant during the blooming seasons of 2016, 2018, and 2019 (17, 3, and 31 days respectively) at LPT, and during 2018 and 2019 (5 and 3 days respectively) at ENP. Observations were carried out on groups of plants with open flowers for 30 min at a time, three times a day. Surveys done between 0700–1200 h were considered to have been performed in the morning, 1800–2019 h in the evening, and 2020–2300 h at night. Nocturnal observations were made using red light lanterns to minimize disturbing the behavior of insect visitors. A total of 75 of these observation periods were conducted in LPT (48 mornings, 20 evenings and 7 nights) on 25 plants, and 11 in ENP (2 mornings, 5 evenings and 4 nights) on 20 plants. Additionally, visitors spotted on flowers of *G. scabra* incidentally while walking through the study sites were recorded. The data reported are the number of observation periods in which each type of visitor was observed.

All arthropods observed touching flowers were considered floral visitors. Due to the potential relevance of lepidopterans in the pollination biology of *G. scabra*, caterpillars feeding on plants were documented, collected, and reared for species determination. Flower visitors were recorded, noting their time of activity and behavior (harvesting reward, contacting sexual organs of the flower, and interacting with other species), and photographed if possible. When necessary, voucher specimens were preserved to confirm identification. These specimens will be deposited in the Florida State Collection of Arthropods (Gainesville, FL, USA).

4.4. Statistical Comparisons

To evaluate the relative importance of different groups of visitors, their behaviors, resources utilized, and activity periods, we used Fisher's exact test (which is appropriate for small sample sizes) to compare the numbers of species associated with each of those parameters. We used a significance level of $p < 0.05$ for single comparisons and $p < 0.01$ for multiple.

5. Conclusions

Although *G. scabra* flowers have traits traditionally associated with attracting nocturnal moths, they open in the evening and remain open into the morning, luring in a much wider array of floral visitors. Despite recent work on the diversity of flower-visiting arthropods in the Everglades [88–90] and pollination of plants in the pine rockland habitat [91–96], little is known about the entire array of flower visitors to any particular plant species. The maintenance of healthy pine rockland habitat requires periodic fires to prevent succession to hardwood hammock forest [84], and in the open pine rockland understory *G. scabra* grow relatively free of competition from other hardwoods, investing much energy into flowering [6]. This study constitutes the first in-depth survey of insects and arachnids associated with the abundant flowers of *G. scabra* in this habitat.

Our findings show that *G. scabr*a is not only visited by nocturnal hawkmoths as expected, but many other potential pollen vectors, beyond those predicted by its pollination syndrome. Our observations also suggest that this plant provides an important foraging and food resource for the local arthropod fauna. Our research provides baseline data on the local arthropod fauna associated with a native plant species, along with insights into the complexity of trophic interactions in the pine rockland habitat. There are 147 recognized species of the genus worldwide [97], but no species of *Guettarda* are considered rare, and those that are ranked by conservation organizations are apparently secure, the habitats in which many occur are imperiled or unranked and threatened in ways similar to the pine rocklands. The richness of floral visitors to *G. scabra* and the critical role this plant may play in sustaining that community indicate that plants may host a wide array of arthropods, regardless of the presence of adaptations suggesting coevolution with a much narrower set of visitors. Our observations on the natural history of *G. scabra* offer a glimpse of how intricate plant-animal interactions can be. For threatened habitats such as the pine rocklands in south Florida, studies like this yield needed information to support efforts to conserve and protect them along with their associated diversity of plants and animals.

Author Contributions

Conceptualization, M.C.P. and S.K.; methodology, M.C.P.; formal analysis, M.C.P.; investigation, M.C.P.; resources, M.C.P. and S.K.; data curation, M.C.P.; writing—original draft preparation, M.C.P.; writing—review and editing, S.K.; visualization, M.C.P.; supervision, S.K.; project ad-ministration, S.K.; funding acquisition, M.C.P. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data that support the findings of this study are openly available in the FIU Research Data Portal at https://doi.org/10.34703/gzx1-9v95/3BRPS3 (accessed on 19 October 2022).

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Waser, N.M.; Chittka, L.; Price, M.V.; Williams, N.M.; Ollerton, J. Generalization in pollination systems, and why it matters. *Ecology* **1996**, *77*, 1043–1060. https://doi.org/10.2307/2265575.

2. Fleming, T.H.; Sahley, C.T.; Holland, J.N.; Nason, J.D.; Hamrick, J.L. Sonoran Desert columnar cacti and the evolution of generalized pollination systems. *Ecol. Monogr.* **2001**, *71*, 511–530. https://doi.org/10.1890/0012-9615071[0511:SDCCAT]2.0.CO;2.

3. de Merxem, D.G.; Borremans, B.; De Jäger, M.L.; Johnson, T.; Jooste, M.; Ros, P.; Zenni, R.D.; Ellis, A.G.; Anderson, B. The importance of flower visitors not predicted by floral syndromes. *South Afr. J. Bot.* **2009**, *75*, 660–667. https://doi.org/10.1016/j.sajb.2009.08.002.

4. Miyake, T.; Yahara, T. Theoretical evaluation of pollen transfer by nocturnal and diurnal pollinators: When should a flower open? *Oikos* **1999**, *86*, 233–240. https://doi.org/10.2307/3546441.

5. Acevedo-Rodríguez, P.; Strong, M.T. *Catalogue of Seed Plants of the West Indies*; Smithsonian Institution: Washington, DC, USA, 2012. https://doi.org/10.5479/si.0081024X.98.1.

6. Koptur, S.; Garcia, D. Habitat differences in morphology and reproductive allocation in *Guettarda scabra* (Rubiaceae). *Castanea* **2017**, *82*, 51–57. https://doi.org/10.2179/16-115.

7. Roberts, A. *Guettarda scabra*. the IUCN Red List of Threatened Species. 2014, e.T56503696A56503850. Available online: https://www.iucnredlist.org/species/56503696/56503850 (accessed on 19 October 2022).

8. WCSP: World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew. 2022. Available online: https://wcsp.science.kew.org (accessed on 19 October 2022).

9. Davis, A.P.; Govaerts, R.; Bridson, D.M.; Ruhsam, M.; Moat, J.; Brummitt, N.A. A global assessment of distribution, diversity, endemism, and taxonomic effort in the Rubiaceae 1. *Ann. Mo. Bot. Gard.* **2009**, *96*, 68–78. https://doi.org/10.3417/2006205.

10. Richards, J.H.; Koptur, S. Floral variation and distyly in *Guettarda scabra* (Rubiaceae). *Am. J. Bot.* **1993**, *80*, 31–40. https://doi.org/10.1002/j.1537-2197.1993.tb13764.x.

11. Faegri, K.; van der Pijl, L. *The Principles of Pollination Ecology*; Pergamon Press: Oxford, UK, 1979.

12. Tomlinson, P.B. *The Biology of Trees Native to Tropical Florida*; Harvard University Printing Office: Allston, MA, USA, 1980.

13. Austin, D.F. *Florida Ethnobotany*; CRC Press: Boca Raton, FL, USA, 2004.

14. Koptur, S. Scientific Note: Insects associated with *Guettarda scabra* in Everglades National Park, Florida. *Castanea* **2020**, *85*, 155–158. https://doi.org/10.2179/0008-7475.85.1.155.

15. Wagner, D.L.; Grames, E.M.; Forister, M.L.; Berenbaum, M.R.; Stopak, D. Insect decline in the Anthropocene: Death by a thousand cuts. *Proc. Natl. Acad.* Sci. *USA* **2021**, *118*, e2023989118. https://doi.org/10.1073/pnas.2023989118.

16. Ruiz, C.; Pimienta, M.C. Behavior of adult *Leucopis* sp. (Chamaemyiidae) associated with aphids feeding on flowers of the rough-leaf velvetseed (*Guettarda scabra*: Rubiaceae) in south Florida. *Fly Times* 2**019**. *62*, 8–10. Available online: https://www.nadsdiptera.org/News/FlyTimes/issue62.pdf (accessed on 19 October 2022).

17. Wardhaugh, C.W. How many species of arthropods visit flowers? *Arthropod-Plant Interact.* **2015**, *9*, 547–565. https://doi.org/10.1007/s11829-015-9398-4.

18. Maruyama, P.K.; Amorim, F.W.; Oliveira, P.E. Night and day service: Distyly and mixed pollination system in *Faramea cyanea* (Rubiaceae). *Flora Morphol. Distrib. Funct. Ecol. Plants* **2010**, *205*, 818–824. https://doi.org/10.1016/j.flora.2010.01.004.

19. Locatelli, E.; Machado, I.C.S. Floral biology of *Cereus fernambucensis*: A sphingophilous cactus of restinga. *Bradleya* **1999**, *17*, 86–94. https://doi.org/10.25223/brad.n17.1999.a5.

20. Walter, H.E. Floral biology of *Echinopsis chiloensis* ssp. *chiloensis* (Cactaceae): Evidence for a mixed pollination syndrome. *Flora Morphol. Distrib. Funct. Ecol. Plants* **2010**, *205*, 757–763. https://doi.org/10.1016/j.flora.2009.12.038.

21. Young, H.J. Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). *Am. J. Bot.* **2002**, *89*, 433–440. https://doi.org/10.3732/ajb.89.3.433.

22. Haber, W.A.; Frankie, G.W. A tropical hawkmoth community: Costa Rican dry forest Sphingidae. *Biotropica* **1989**, *21*, 155–172. https://doi.org/10.2307/2388706.

23. Miyake, T.; Yahara, T. Why does the flower of *Lonicera japonica* open at dusk? *Can. J. Bot.* **1998**, *76*, 1806–1811. https://doi.org/10.1139/b98-119.

24. Darrault, R.O.; Schlindwein, C. Esfingídeos (Lepidoptera, Sphingidae) no Tabuleiro Paraibano, nordeste do Brasil: Abundância, riqueza e relação com plantas esfingófilas. *Rev. Bras. De Zool.* **2002**, *19*, 429–443. https://doi.org/10.1590/S0101-81752002000200009.

25. Aguilar-Rodríguez, P.A.; Krömer, T.; García-Franco, J.G.; MacSwiney, G.M.C. From dusk till dawn: Nocturnal and diurnal pollination in the epiphyte *Tillandsia heterophylla* (Bromeliaceae). *Plant Biol.* **2016**, *18*, 37–45. https://doi.org/10.1111/plb.12319.

26. Wolff, D.; Braun, M.; Liede, S. Nocturnal versus diurnal pollination success in *Isertia laevis* (Rubiaceae): A sphingophilous plant visited by hummingbirds. *Plant Biol.* **2003**, *5*, 71–78. https://doi.org/10.1055/s-2003-37977.

27. Xu, Y.; Luo, Z.; Gao, S.; Zhang, D. Pollination niche availability facilitates colonization of *Guettarda speciosa* with heteromorphic self-incompatibility on oceanic islands. *Sci. Rep.* **2018**, *8*, 13765. https://doi.org/10.1038/s41598-018-32143-5.

28. Martínez, L. Fenología reproductiva y efecto del robo de néctar en el éxito reproductivo de *Guettarda clarensis*, en. Bachelor’s Thesis, Universidad Central “Marta Abreu” de Las Villas, Santa Clara, Villa Clara, Cuba, 2013. Available online: https://dspace.uclv.edu.cu/handle/123456789/1723 (accessed on 19 October 2022).

29. Novo, R.R.; Consolaro, H.; Almeida, N.M.; Castro, C.C. Floral biology of the velvetseed *Guettarda platypoda* DC. (Rubiaceae): Atypical distyly or style dimorphism? *Flora Morphol. Distrib. Funct. Ecol. Plants* **2018**, *239*, 62–70. https://doi.org/10.1016/j.flora.2017.11.008.

30. Warriner, M.D. A range extension for the large carpenter bee *Xylocopa micans* (Hymenoptera: Apidae) with notes on floral and habitat associations. *J. Kans. Entomol. Soc.* **2010**, *83*, 267–269. https://doi.org/10.2317/JKES0910.14.1.

31. Lenczewski, B. *Butterflies of Everglades National Park*. Report T-588; South Florida Research Center, Homestead, Florida, USA: 1980; 110p.

32. Fishbein, M.; Venable, D.L. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* **1996**, *77*, 1061–1073. https://doi.org/10.2307/2265576.

33. Watanabe, K.; Kato, H.; Kuraya, E.; Sugawara, T. Pollination and reproduction of *Psychotria homalosperma*, an endangered distylous tree endemic to the oceanic Bonin (Ogasawara) Islands, Japan. *Plant Species Biol.* **2018**, *33*, 16–27. https://doi.org/10.1111/1442-1984.12183.

34. Pemberton, R.W.; Wheeler, G.S. Orchid bees don't need orchids: Evidence from the naturalization of an orchid bee in Florida. *Ecology* **2006**, *87*, 1995–2001. https://doi.org/10.1890/0012-965887%5B1995:OBDNOE%5D2.0.CO;2.

35. Koptur, S. Flowering phenology and floral biology of *Inga* (Fabaceae: Mimosoideae). *Syst. Bot.* **1983**, *8*, 354–368. https://doi.org/10.2307/2418355.

36. Koptur, S.; Peña, S.; Barrios R.B. Do morning butterfly visitors benefit a night-flowering hawkmoth pollinated plant? *Castanea* **2021**, *86*, 100–111. https://doi.org/10.2179/0008-7475.86.1.100.

37. Genini, J.; Morellato, L.P.C.; Guimarães, P.R., Jr.; Olesen, J.M. Cheaters in mutualism networks. *Biol. Lett.* **2010**, *6*, 494–497. https://doi.org/10.1098/rsbl.2009.1021.

38. Martínez-Pérez, L.; Faife-Cabrera, M. Nectar robbing in *Guettarda clarensis* (Rubiaceae): Does floral neighborhood matter? *Rev. Del. Jardín Botánico Nac.* **2019**, *40*, 47–57. Available online: https://www.rjbn.uh.cu/index.php/RJBN/article/view/443/465 (accessed on 19 October 2022).

39. Martínez, L. Relación del Vecindario Floral Con la Frecuencia de Robo de Néctar en *Guettarda clarensis* (Rubiaceae). Master’s Thesis, Universidad Central “Marta Abreu” de Las Villas, Santa Clara, Villa Clara, Cuba, 2017. Available online: https://dspace.uclv.edu.cu/handle/123456789/10813 (accessed on 19 October 2022).

40. Schlindwein, C.; Westerkamp, C.; Carvalho, A.T.; Milet-Pinheiro, P. Visual signalling of nectar-offering flowers and specific morphological traits favour robust bee pollinators in the mass-flowering tree *Handroanthus impetiginosus* (Bignoniaceae). *Bot. J. Linn. Soc.* ***2014****, 176, 396–407.*

41. Irwin, R.E.; Bronstein, J.L.; Manson, J.S.; Richardson, L. Nectar robbing: Ecological and evolutionary perspectives. *Annu. Rev. Ecol. Evol. Syst.* **2010**, *41*, 271–292. https://doi.org/10.1146/annurev.ecolsys.110308.120330.

42. Barrows, E.M. Robbing of exotic plants by introduced carpenters and honeybees in Hawaii, with comparative notes. *Biotropica* **1980**, *12*, 23–29. https://doi.org/10.2307/2387770.

43. Dedej, S.; Delaplane, K.S. Nectar-robbing carpenter bees reduce seed-setting capability of honey bees (Hymenoptera: Apidae) in rabbiteye blueberry, *Vaccinium ashei*, ‘Climax’. *Environ. Entomol.* **2004**, *33*, 100–106. https://doi.org/10.1603/0046-225X-33.1.100.

44. Guitián, J.; Sánchez, J.M.; Guitián, P. Pollination ecology of *Petrocoptis grandiflora* Rothm. (Caryophyllaceae); a species endemic to the north-west part of the Iberian Peninsula. *Bot. J. Linn. Soc.* **1994**, *115*, 19–27.

45. Schmid, S.; Schmid, V.S.; Zillikens, A.; Steiner, J. Diversity of flower visitors and their role for pollination in the ornithophilous bromeliad *Vriesea friburgensis* in two different habitats in southern Brazil. *Ecotropica* **2011**, *17*, 91–102.

46. Zhang, Y.W.; Robert, G.W.; Wang, Y.; Guo, Y.H. Nectar robbing of a carpenter bee and its effects on the reproductive fitness of *Glechoma longituba* (Lamiaceae). *Plant Ecol.* **2007**, *193*, 1–13. https://doi.org/10.1007/s11258-006-9244-y.

47. Scott, P.E.; Buchmann, S.L.; O'Rourke, M.K. Evidence for mutualism between a flower-piercing carpenter bee and ocotillo: Use of pollen and nectar by nesting bees. *Ecol. Entomol.* **1993**, *18*, 234–240. https://doi.org/10.1111/j.1365-2311.1993.tb01095.x.

48. Gottsberger, G. Some pollination strategies in neotropical savannas and forests. *Plant Syst. Evol.* **2004**, *152*, 29–45.

49. Singh, V.K.; Barman, C.; Tandon, R. Nectar Robbing Positively Influences the Reproductive Success of *Tecomella undulata* (Bignoniaceae). *PLoS ONE* **2014**, *9*, e102607. https://doi.org/10.1371/journal.pone.0102607.

50. Maloof, J. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. *Am. J. Bot.* **2001**, *88*, 1960–1965.

51. Loope, L.L. *Phenology of Flowering and Fruiting in Plant Communities of Everglades National Park and Biscayne National Monument, Florida*; Report T-593; South Florida Research Center, Homestead, Florida, USA: 1980; 50p.

52. Gunderson, L.; Taylor, D.; Craig, J. *Fire Effects on Flowering and Fruiting Patterns of Understory Plants in Pinelands of Everglades National Park*; Report SFRC-83/04; South Florida Research Center, Homestead, Florida, USA: 1983; 36p.

53. Grissell, E.E.*Mason Wasps of Florida, Zethus spp. (Insecta: Hymenoptera: Vespidae: Eumeninae*); Entomology Circular 153; Florida Department of Agriculture and Consumer Services, Division of Plant Industry, UF/IFAS Extension: Gainesville, FL, USA, 2021; pp. 1–3.

54. Grissell, E.E. *Scoliid Wasps of Florida, Campsomeris, Scolia and Trieliss pp. (Insecta: Hymenoptera: Scoliidae*); Entomology Circular 179 and 185; Florida Department of Agriculture and Consumer Services, Division of Plant Industry, UF/IFAS Extension: Gainesville, FL, USA, 2017; pp. 1–14.

55. Tuttle, J.P. *The Hawk Moths of North America: A Natural History Study of the Sphingidae of the United States and Canada*; Wedge Entomological Research Foundation: Washington, DC, USA, 2007.

56. Warren, A.D.; Davis, K.J.; Grishin, N.V.; Pelham, J.P.; Stangeland, E.M. Interactive Listing of American Butterflies. 2012. Available online: https://www.butterfliesofamerica.com/ (accessed on 19 October 2022).

57. Daniels, J. Florida butterflies. *Wings: Essays Invertebr. Conserv.* **2010**, *33*, 18–21. Available online: https://xerces.org/wings (accessed on 19 October 2022).

58. Boggs, C.L. Nutritional and life-history determinants of resource allocation in holometabolous insects. *Am. Nat.* **1981**, *117*, 692–709. https://doi.org/10.1086/283753.

59. Gilbert, L.E. Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proc. Natl. Acad. Sci. USA* **1972**, *69*, 1403–1407. https://doi.org/10.1073/pnas.69.6.1403.

60. O'Brien, D.M.; Boggs, C.L.; Fogel, M.L. Pollen feeding in the butterfly *Heliconius charitonia*: Isotopic evidence for essential amino acid transfer from pollen to eggs. *Proc. R. Soc. London. Ser. B Biol. Sci.* **2003**, *270*, 2631–2636.

61. Slotten, J.R.; Miller, W. Occurrence of *Eupyrrhoglossum sagra* and *Perigonia lusca* in Florida (Lepidoptera: Sphingidae). *Holarct. Lepid.* **2000**, *7*, 59–63.

62. Thomas, M.C. *A Flower Beetle, Euphoria Sepulcralis (Fabricius) (Insecta: Coleoptera: Scarabaeidae*); Florida Department of Agriculture and Consumer Services, Division of Plant Industry, UF/IFAS Extension, Gainesville, Florida, USA. Entomology Circular 386: 2019; pp. 1–6.

63. Jackson, R.R.; Pollard, S.D.; Nelson, X.J.; Edwards, G.B.; Barrion, A.T. Jumping spiders (Araneae: Salticidae) that feed on nectar. *J. Zool.* **2001**, *255*, 25–29. https://doi.org/10.1017/S095283690100108X.

64. Marquínez, X.; Cepeda, J.; Lara, K.; Sarmiento, R. Arañas asociadas a la floración de *Drimys granadensis* (Winteraceae). *Rev. Colomb. De Entomol.* **2010**, *36*, 172–175.

65. Nahas, L.; Gonzaga, M.O.; Del-Claro, K. Wandering and web spiders feeding on the nectar from extrafloral nectaries in neotropical savanna. *J. Zool.* **2017**, *301*, 125–132. https://doi.org/10.1111/jzo.12400.

66. Nyffeler, M.; Olson, E.J.; Symondson, W.O. Plant-eating by spiders. *J. Arachnol.* **2016**, *44*, 15–27. https://doi.org/10.1636/P15-45.1.

67. Taylor, R.M.; Foster, W.A. Spider nectarivory. *Am. Entomol.* **1996**, *42*, 82–86. https://doi.org/10.1093/ae/42.2.82.

68. Vogelei, A.; Greissl, R. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). *Oecologia* **1989**, *80*, 513–515. https://doi.org/10.1007/BF00380075.

69. Dukas, R. Effects of perceived danger on flower choice by bees. *Ecol. Lett.* **2001**, *4*, 327–333. https://doi.org/10.1046/j.1461-0248.2001.00228.x.

70. Morse, D.H. Choice of hunting site as a consequence of experience in late-instar crab spiders. *Oecologia* **1999**, *120*, 252–257. https://doi.org/10.1007/s004420050855.

71. Dukas, R.; Morse, D.H. Crab spiders affect flower visitation by bees. *Oikos* **2003**, *101,* 157–163. https://doi.org/10.1034/j.1600-0706.2003.12143.x.

72. Llandres, A.L.; De Mas, E.; Rodriguez-Girones, M.A. Response of pollinators to the tradeoff between resource acquisition and predator avoidance. *Oikos* **2012**, *121,* 687–696. https://doi.org/10.1111/j.1600-0706.2011.19910.x.

73. Robertson, I.C.; Maguire, D.K. Crab spiders deter insect visitations to slickspot peppergrass flowers. *Oikos* **2005**, *109,* 577–582. https://doi.org/10.1111/j.0030-1299.2005.13903.x.

74. Romero, G.Q.; Antiqueira, P.A.; Koricheva, J. A meta-analysis of predation risk effects on pollinator behaviour. *PLoS ONE* **2011**, *6*, e20689. https://doi.org/10.1371/journal.pone.0020689.

75. Suttle, K.B. Pollinators as mediators of top-down effects on plants. *Ecol. Lett.* **2003**, *6,* 688–694. https://doi.org/10.1046/j.1461-0248.2003.00490.x.

76. Gentry, A.H. Anti-Pollinators for Mass-Flowering Plants? *Biotropica* **1978**, *10,* 68–69. https://doi.org/10.2307/2388108.

77. Hopkins, H.C.; Hopkins, M.J. Predation by a snake of a flower-visiting bat at *Parkia nitida* (Leguminosae: Mimosoideae). *Brittonia* **1982**, *34*, 225–227.

78. Romero, G.Q.; Vasconcellos-Neto, J. Beneficial effects of flower-dwelling predators on their host plant. *Ecology* **2004**, *85,* 446–457. https://doi.org/10.1890/02-0327.

79. Carpenter, J.M. The genus *Pachodynerus* in North America (Hymenoptera: Vespidae: Eumeninae). *Proc. Entomol. Soc. Wash.* **1986**, *88*, 572–577.

80. Krombein, K.V. *Trap-Nesting Wasps and Bees: Life Histories, Nests and Associates*; Smithsonian Press: Washington, DC, USA, 1967.

81. Bradley, J.C. The species of *Campsomeris* (Hymenoptera-Scoliidae) of the Plumipes Group, inhabiting the United States, the Greater Antilles, and the Bahama Islands. *Proc. Acad. Sci. Phila.* **1928**, *80*, 313–337.

82. Florida Natural Areas Inventory (FNAI). Pine Rockland. In *Guide to the Natural Communities of Florida*; Florida Natural Areas Inventory: Tallahassee, FL, USA, 2010; pp. 69–72.

83. Koptur, S. The conservation of specialized and generalized pollination systems in subtropical ecosystems: A case study. In *Plant–Pollinator Interactions: From Specialization to Generalization*; Waser, N., Ollerton, J., Eds.; University of Chicago Press: Chicago, IL, USA, 2006; pp. 341–361.

84. Snyder, J.R.; Herndon, A.; Robertson, W.B., Jr. South Florida Rockland. In *Ecosystems of Florida*; Myers, R.L., Ewel, J.J., Eds.; University of Central Florida Press: Orlando, FL, USA, 1990; pp. 230–274.

85. Peña, A.L.; Koptur, S. A Historical Floristic Inventory of Pine Rockland Fabaceae (Leguminosae). *Nat. Areas J.* **2021**, *41*, 258–272. https://doi.org/10.3375/20-25.

86. Possley, J.E.; Maschinski, J.M.; Maguire, J.; Guerra, C. Vegetation Monitoring to Guide Management Decisions in Miami's Urban Pine Rockland Preserves. *Nat. Areas J.* **2014**, *34*, 154–165. https://doi.org/10.3375/043.034.0205.

87. Natureserve. 2022. Available online: https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.723149/South_Florida_Pine_Rockland (accessed on 19 October 2022).

88. Artz, D.R.; Waddington, K.D. The Effects of Neighbouring Tree Islands on Pollinator Density and Diversity, and on Pollination of a Wet Prairie Species, *Asclepias lanceolata* (Apocynaceae). *J. Ecol.* **2006**, *94*, 597–608.

89. Pascarella, J.B.; Waddington, K.D.; Neal, P.R. The Bee Fauna (Hymenoptera: Apoidea) of Everglades National Park, Florida and Adjacent Areas: Distribution, Phenology, and Biogeography. *J. Kans. Entomol. Soc.* **1999**, *72*, 32–45.

90. Pascarella, J.B.; Waddington, K.D.; Neal, P.R. Non-apoid flower-visiting fauna of Everglades National Park, Florida. *Biodivers. Conserv.* **2001**, *10*, 551–566.

91. Pascarella, J.B. Pollination Ecology of *Ardisia escallonioides* (Myrsinaceae). *Castanea* **1997**, 62, 1–7.

92. Pascarella, J.B. Hurricane Disturbance, Plant-Animal Interactions, and the Reproductive Success of a Tropical Shrub. *Biotropica* **1998**, *30*, 416–424.

93. Cardel, Y.; Koptur, S. Effects of Florivory on the Pollination of Flowers: An Experimental Field Study with a Perennial Plant. *Int. J. Plant Sci.* **2010**, *171*, 283–292.

94. Harris, B.M.; Koptur, S. Facilitated fecundity in sand flax: Pollination in an endangered herb of pine rocklands. *Flora* **2022**, *289*, 152041. https://doi.org/10.1016/j.flora.2022.152041.

95. Linares, L.J.; Koptur, S. Floral Biology and Breeding System of the Crenulate Leadplant, *Amorpha herbacea* var. *crenulata*, an Endangered South Florida Pine Rockland Endemic. *Nat. Areas J.* **2010**, *30*, 138–147. https://doi.org/10.3375/043.030.0202.

96. Liu, H.; Koptur, S. Breeding System and Pollination of a Narrowly Endemic Herb of the Lower Florida Keys: Impacts of the Urban Wildland Interface. *Am. J. Bot.* **2003**, *90*, 1180–1187.

97. Royal Botanic Gardens, Kew 2021. The World Checklist of Vascular Plants (WCVP). Checklist dataset. https://doi.org/10.15468/6h8ucr (accessed on 19 October 2022).

Tables

Table 1. Array of arthropods associated with *Guettarda scabra*, their behaviors, and plant resources used at two pine rockland sites (Larry and Penny Thompson Memorial Park: LPT; and Long Pine Key, Everglades National Park: ENP) in south Florida. Observed behaviors abbreviated as follows: predation on other arthropods (pr), legitimate visitation (lv), primary nectar robbing (1nr), secondary nectar robbing (2nr), and herbivory (h). Plant resources used by visitor abbreviated as follows: arthropod prey (ap), nectar (n), pollen (p), floral tissue (f), and leaves (l). Asterisks signify caterpillar stage.

CLASS ORDER Family Species (Author)	**Behavior on Plant**	**Resource Used**	**Study Site**	
			LPT	ENP
ARACHNIDA				
ARANEAE				
Araneidae				
Acacesia hamata Hentz	pr	ap	x	
Thomisidae				
Mecaphesa sp. 1	pr	ap	x	
Mecaphesa sp. 2	pr	ap	x	
Mecaphesa sp. 3	pr	ap	x	
Mecaphesa sp. 4	pr	ap	x	
INSECTA				
BLATTODEA				
Blattodea sp.1	2nr	n	x	
COLEOPTERA				
Cerambycidae				
Eburia stigma Oliver	lv	p		x
Plectromerus dentipes Oliver	lv	p		x
Scarabaeidae				
Euphoria sepulcralis Fabricius	lv	p	x	
Phyllophaga sp.	h	f	x	
DIPTERA				
Syrphidae				
Ornidia obesa Fabricius	lv	p	x	
Tipulidae				
Tipulidae sp.1	2nr	n	x	
Tipulidae sp.2	2nr	n		x
HEMIPTERA				
Aphididae				
Aphididae sp.1	h	f	x	
Largidae				

CLASS **ORDER** **Family** **Species (Author)**	**Behavior on Plant**	**Resource Used**	**Study Site**	
			LPT	ENP
Largus succinctus Linnaeus	2nr	n	x	x
HYMENOPTERA				
Apidae				
Apis mellifera Linnaeus	2nr	n		x
Euglosssa dilemma Bembé & Eltz	lv	n		x
Xylocopa micans Lepeletier	1nr	n		x
Crabronidae				
Cerceris rufopicta Smith	2nr	n	x	
Formicidae				
Camponotus floridanus Buckley	2nr	n	x	
Pseudomyrmex gracilis Fabricius	2nr	n	x	
Wasmannia auropunctata Roger	2nr	n	x	
Halictidae				
Augochloropsis sp.	2nr	n		x
Scoliidae				
Dielis trifasciata Fabricius	lv	p	x	
Vespidae				
Mischocyttarus mexicanus cubicola Richards	2nr	n	x	
Pachodynerus erynnis Lepeletier	2nr	n	x	
Stenodynerus sp.	1nr, 2nr	n	x	
Vespidae sp.1	1nr	n		x
Zethus slossonae Fox	1nr	n	x	x
LEPIDOPTERA				
Erebidae				
Calidota laqueata Edwards *	h	l	x	x
Hypercompe scriboni Stoll *	h	l	x	
Seirarctia echo Smith *	h	l	x	
Hesperiidae				
Asbolis capucinus Lucas	lv	n	x	x
Cymaenes tripunctus Herrich-Schäffer	lv	n	x	
Ephyriades brunnea Herrich-Schäffer	lv	n	x	x
Polites baracoa Lucas	lv	n	x	
Nymphalidae				
Agraulis vanillae Linnaeus	lv	n	x	x
Heliconius charithonia Linnaeus	lv	n	x	
Papilionidae				
Papilio palamedes Drury	lv	n		x

CLASS **ORDER** **Family** **Species (Author)**	**Behavior on Plant**	**Resource Used**	**Study Site**	
			LPT	ENP
Papilio polyxenes Fabricius	lv	n	x	
Sphingidae				
Aellopos tantalus Linnaeus	lv	n	x	
Eumorpha fasciatus Sulzer	lv	n		x
Perigonia lusca Fabricius	lv	n		x
Xylophanes tersa Linnaeus	lv	n	x	x
MANTODEA				
Mantidae				
Mantidae sp.1	pr	ap	x	
Stagmomantis floridensis Davis	pr	ap	x	

Figures

Figure 1. (a) Recently opened flowers of *Guettarda scabra*, during late afternoon. Some individuals, such as the one in this picture, have a long pistil that raises the stigma above the deep corolla tube. Exudates from the stigma were occasionally consumed by visitors such as flies, beetles, and possibly spiders during this study; (b) general view of pine rockland habitat at Long Pine Key, Everglades National Park in south Florida, USA. *Guettarda scabra* plants are abundant in patches scattered among *Pinus elliottii* trees.

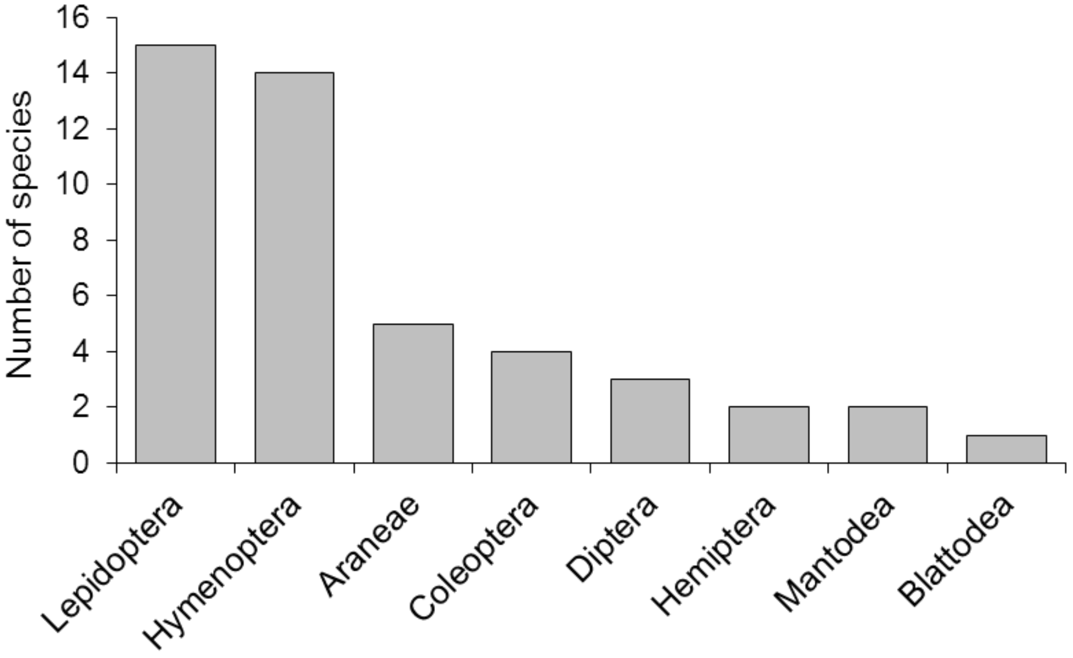

Figure 2. Arthropod orders observed on flowers of *Guettarda scabra*, sorted according to the number of species found. The large numbers of lepidopterans and hymenopterans are mostly related to diurnal activity in these two groups.

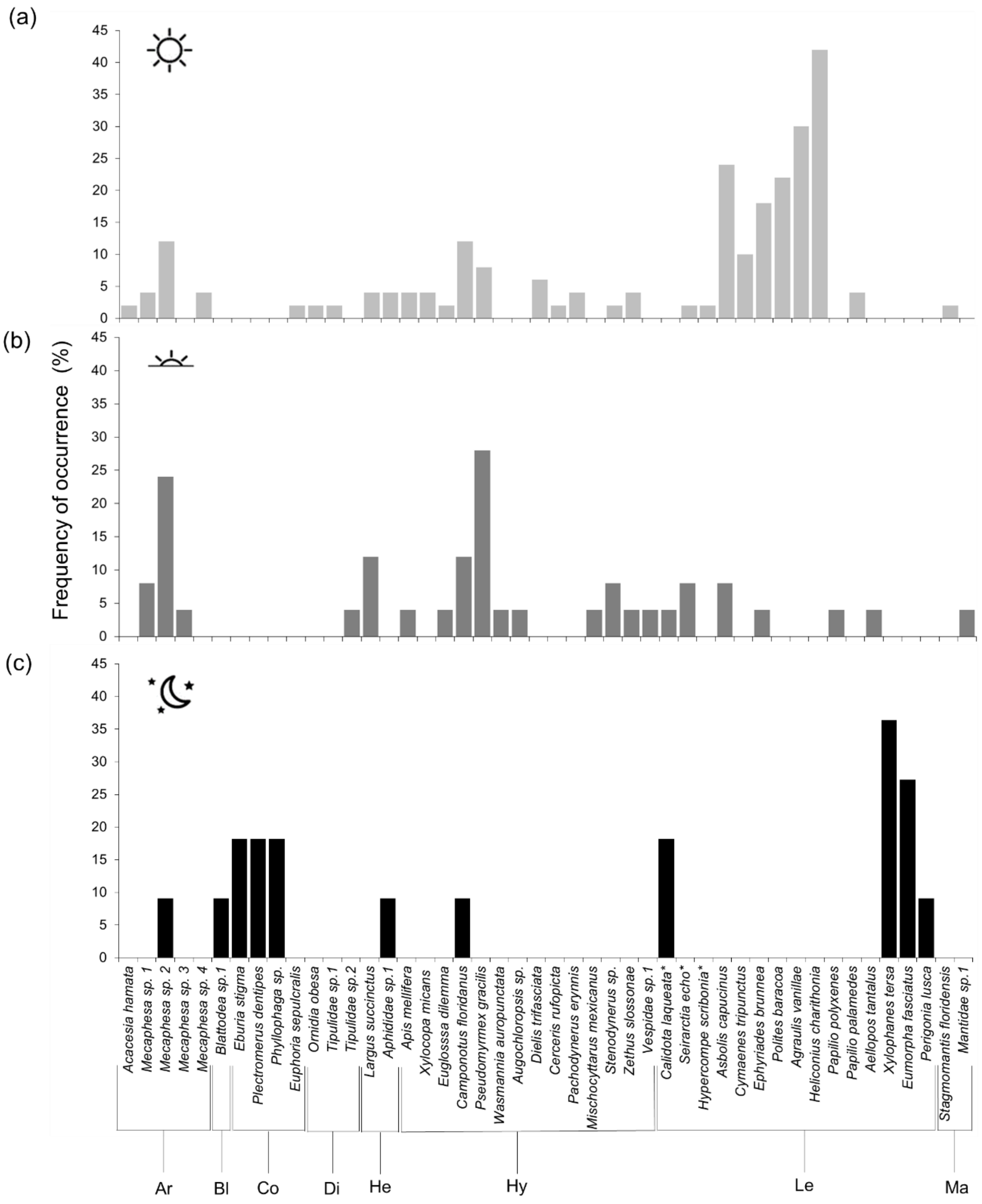

Figure 3. Frequency of occurrence of each visitor species on flowers of *Guettarda scabra*, relative to the total number of observation periods carried on during the (a) morning (N = 50), (b) evening (N = 25), or (c) night (N = 11). Ar: Araneae; Bl: Blattodea; Co: Coleoptera; Di: Diptera; He: Hemiptera; Hy: Hymenoptera; Le: Lepidoptera; Ma: Mantodea. Asterisks (*) refer to caterpillars.

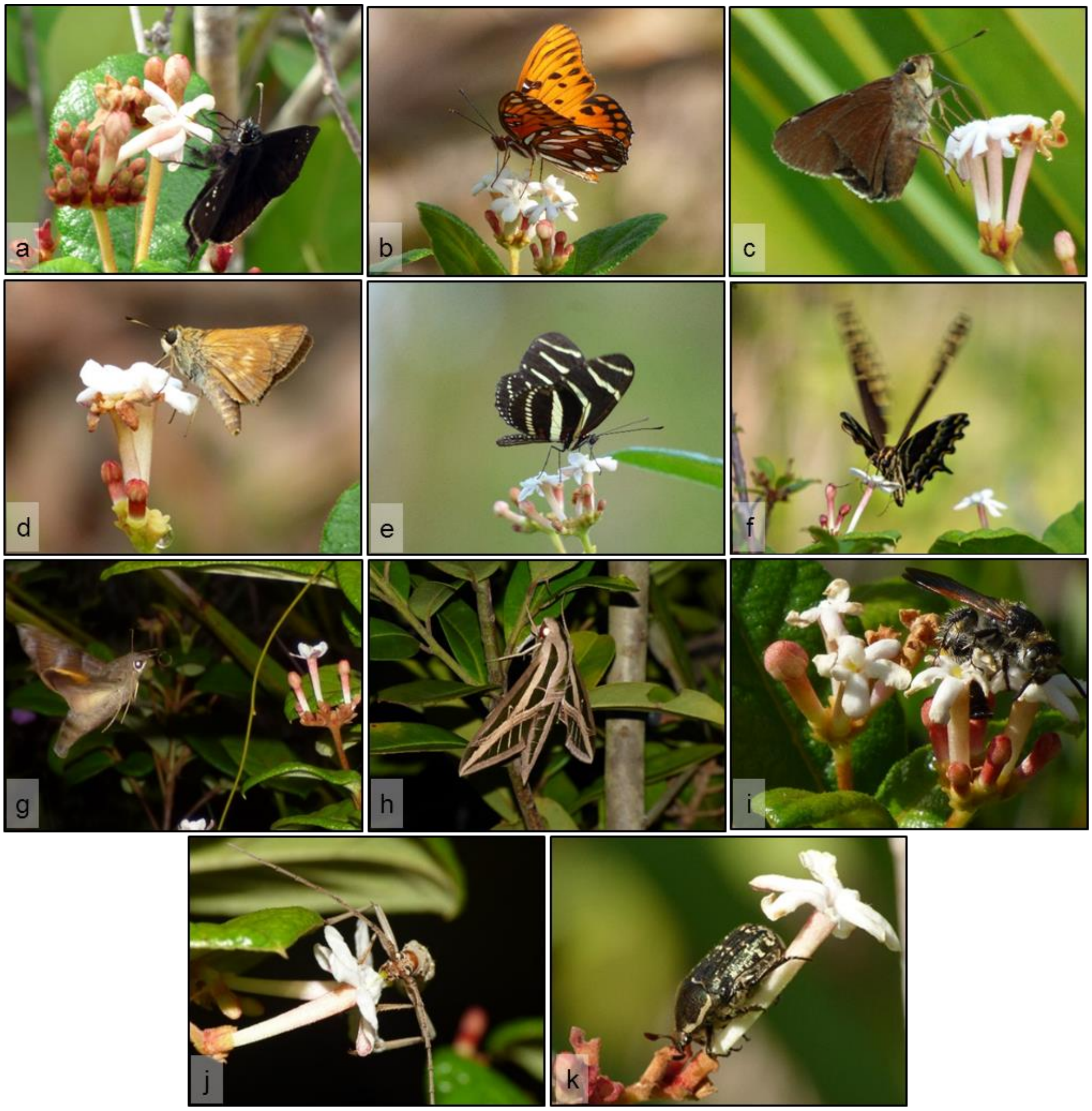

Figure 4. Overview of some species of flower visitors performing legitimate visitation and behaving as potential pollinators of *Guettarda scabra* at two pine rockland sites (Larry and Penny Thompson Memorial Park, LPT; and Long Pine Key, Everglades National Park, ENP) in south Florida, USA. Some lepidopterans such as (a) *Ephyriades brunnea*, (b) *Agraulis vanillae*, and (c) *Asbolis capucinus* were observed in both study sites, while (d) *Polites baracoa* and (e) *Heliconius charithonia* were seen only in LPT. Other visitors were only seen in ENP, such as (f) *Papilio palamedes* that feeds on nectar during daytime, and the nocturnal hawkmoths (g) *Perigonia lusca* and (h) *Eumorpha fasciatus*, represented here by an individual resting after a feeding bout. Besides lepidopterans, (i) the wasp *Dielis trifasciata* is seen here coming in close contact with the exposed stigma of a flower as it feeds on pollen during the morning. Beetles such as (j) *Eburia stigma* and (k) *Euphoria sepulcralis* visited flowers to feed on pollen and stigma exudates.

Figure 5. Overview of nectar robbers found on flowers of *Guettarda scabra* in pine rockland habitats in south Florida, USA. Diurnal primary nectar robbers such as the wasps (a) *Zethus slossonae*, (b) *Stenodynerus* sp., and the bee (c) *Xylocopa micans* use their mandibles to pierce the base of the corolla to access the nectar. Notice how the hairy underside of the abdomen in *X. micans* comes in close contact with the stigma of the flower, as the bee cuts the corolla tissue, potentially leading to pollen transfer. Secondary nectar robbers such as the honeybee (d) *Apis mellifera*, the ants (e) *Pseudomyrmex gracilis* and (f) *Camponotus floridanus*, the true bug (g) *Largus succinctus* (nymph), and (h) a cockroach (*Blattodea* sp.), drink nectar through holes cut at the base of the corolla tube by a previous visitor. Opportunistic ant visitors such as *P. gracilis* can also feed on nectar from postfloral nectaries (i) or as observed in *C. floridanus*, feed on honeydew secreted by aphids (j).

Figure 6. Overview of predatory and herbivorous arthropods on *Guettarda scabra* in pine rockland habitats in south Florida, USA. (a) Crab spiders of the genus *Mecaphesa* in hunting position on a corolla, and (b) on an unopened bud. (c) Orbweaver spider *Acacesia hamata* sitting on an open flower almost touching the exposed stigma. (d) Praying mantis *Stagmomantis floridensis* exploring a branch in the morning. Caterpillars of the erebid moths (e) *Calidota laqueata*, (f) *Hypercompe scribonia*and, and (g) *Seirarctia echo*, found consuming leaves of *G. scabra*. Other herbivores found associated with flowers include (h) clusters of aphids sucking sap from a flower bud, and a (i) May beetle *Phyllophaga* sp. chewing on a flower bud at night.

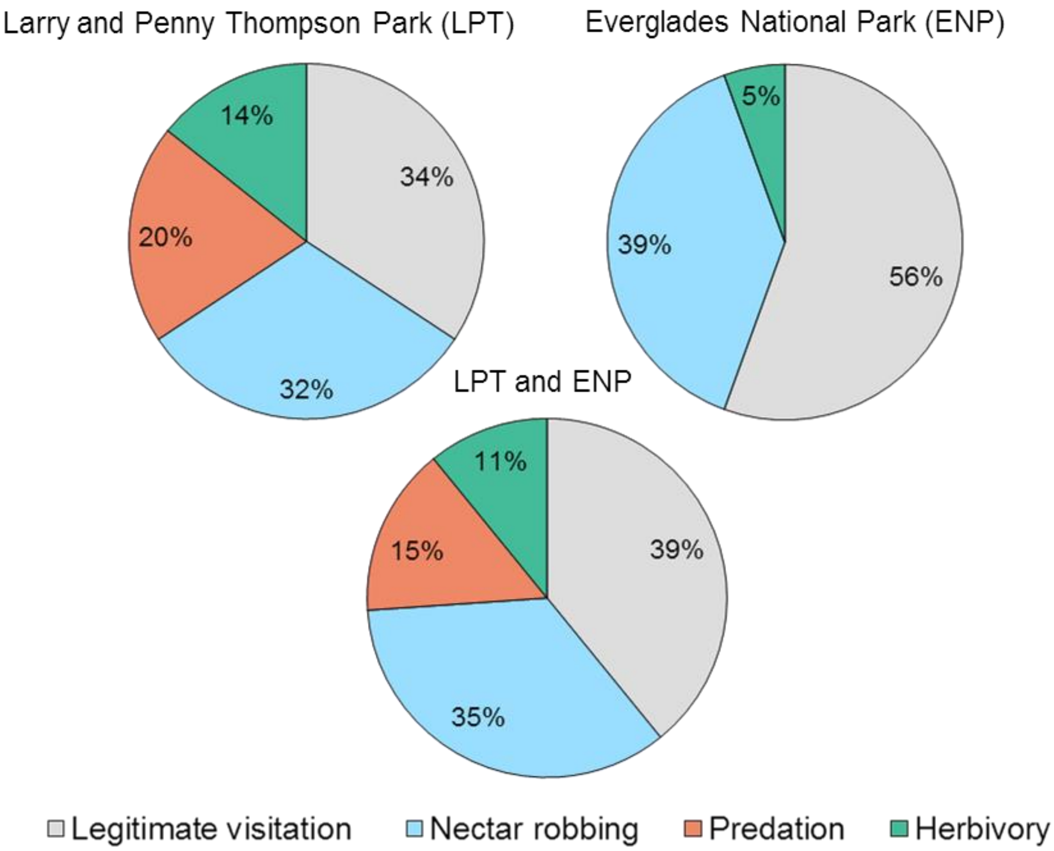

Figure 7. Relative occurrence of the four visitor behaviors observed on flowers of *Guettarda scabra*, among arthropod species in pine rockland habitats in south Florida, USA. Percentages represent the fraction of species observed engaging in a particular behavior on flowers, with respect to the total number of species found in Larry and Penny Thompson LPT (35 species), in Everglades National Park ENP (18 species), or in both sites combined (46 species).

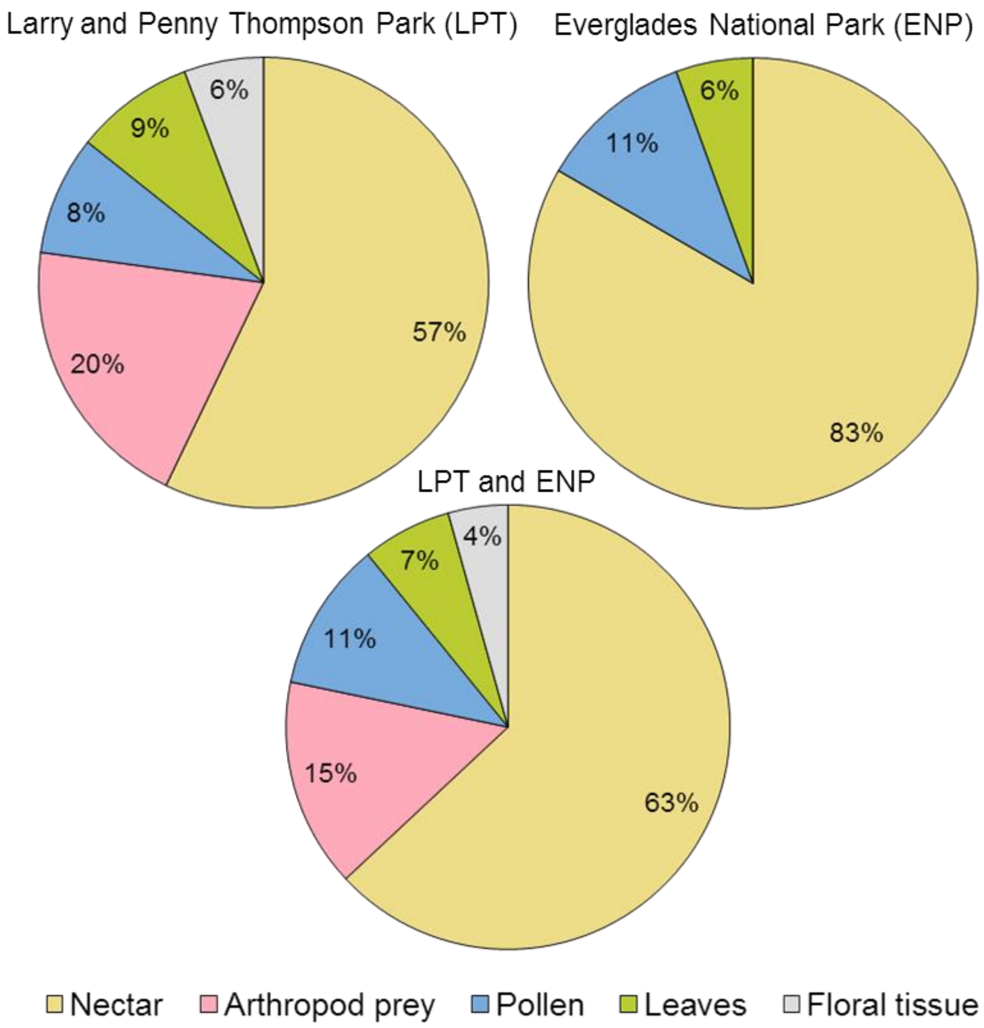

Figure 8. Relative usage of plant resources provided by *Guettarda scabra*, among arthropods in pine rockland habitats in south Florida USA. Percentages represent the fraction of species benefiting from a particular resource, with respect to the total number of species found in Larry and Penny Thompson LPT (35 species), in Everglades National Park ENP (18 species), or in both sites combined (46 species).

CHAPTER III

DEPENDENCE ON NOCTURNAL POLLINATORS DESPITE MOST FLOWER VISITATION OCCURRING DURING THE DAY: THE CASE OF A NIGHT-BLOOMING PLANT IN SOUTH FLORIDA PINE ROCKLANDS.

María Cleopatra Pimienta[1], Carlos Ruiz[2], and Suzanne Koptur[1,]

[1]Department of Biological Sciences, Florida International University, 11200 S.W. 8th Street, Miami, FL 33199 USA.

[2]Department of Biology, University of Washington, Seattle, WA, 98195, USA

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Abstract

Premise: A large number of angiosperms rely on insects for sexual reproduction, without their pollinators many would go extinct. In southern Florida, the rough-leaf velvetseed, *Guettarda scabra* L. (Vent.), exists only in remnants of the imperiled pine rockland habitat. This species exhibits all the floral traits typical for attracting nocturnal hawkmoths (Sphingidae), but is also abundantly visited by diurnal lepidopterans, raising questions of whether the sphingophilous nature of this plant represents actual dependence on nocturnal visitors for its reproduction.

Methods: We evaluated the relative contribution of diurnal and nocturnal flower visitors to female reproductive success by selectively exposing flowers of *G. scabra* to these groups. We also determined the identity of all lepidopteran flower visitors by direct observation during a flowering season in a natural population in south Florida, US.

Results: We found that flowers of *G. scabra* receive legitimate visits by both diurnal and nocturnal lepidopterans in a suburban pine rockland fragment in south Florida. Excluding diurnal visitors had no detrimental effect on the plant's reproduction, while restricting access to nocturnal visitors drastically reduced fruit-set. Plant fruit-set mediated by nocturnal pollinators was significantly higher than by diurnal visitors, although the daytime visitors were more frequent.

Conclusions: Regardless of the frequent visitation by diurnal lepidopterans, nocturnal hawkmoths are required for successful sexual reproduction in *G. scabra*, suggesting a specialized pollination system. Our findings show that the nocturnal moth *Xylophanes tersa* is the most likely effective pollinator for *G. scabra* locally, while diurnal visitors act exclusively as nectar and pollen thieves.

Keywords: diurnal; flower visitor; exclusion experiment; fruit set; *Guettarda scabra*; hawkmoths; Lepidoptera; pollinator; nocturnal; Rubiaceae.

1. Introduction

Floral traits in animal-pollinated plants are usually the result of the interaction between plants and their pollinators (Baker and Hurd, 1968; Willmer, 2011). However, even when a plant exhibits some degree of floral specialization associated with a particular group of pollinators, it often receives visits from a broader spectrum of flower visitors, some of which can contribute to its successful reproduction (Fishbein and Venable, 1996; Miyake and Yahara, 1998; de Merxem et al., 2009), while others play a minor role, or none at all (e.g., Gribel and Hay, 1993; Castro et al., 2013; Martins et al., 2016).

Plants with sphingophilous flowers exhibit traits associated with hawkmoth pollination (Lepidoptera: Sphingidae), such as a white tubular corolla, strong sweet fragrance and anthesis at dusk (Faegri and van der Pijl, 1979). Flowers of these plants can remain open and available to visitation into the following day even though their presumably coevolved pollinators are not active at that time (e.g., Young, 2002; Wolff et al., 2003; Aguilar-Rodríguez et al., 2016; Danaher et al., 2020).

The rough-leaf velvetseed, *Guettarda scabra* (L.) Vent. (Rubiaceae), is a neotropical hardwood shrub with flowers suggesting hawkmoth pollination that remain open until midmorning the next day (Koptur, 2020), hence being exposed to diurnal visitors. Regardless of its sphingophilous morphology, *G. scabra* flowers are visited by a diverse array of diurnal and nocturnal arthropods, with Lepidoptera being the main group performing legitimate visits and presumably contributing to pollination in this plant (Pimienta and Koptur, 2022). However, not much is known about the actual role of these lepidopteran visitors in the reproduction of *G. scabra*.

We set out to identify diurnal and nocturnal lepidopteran visitors and evaluate their relative contribution to the female reproductive success of *G. scabra*. By selectively exposing plants to these two groups of insects, we determined their role in the pollination and fruit production in this plant and identified the most likely effective pollinators for this species. Given that anthesis in *G. scabra* is mainly nocturnal, we expected night-active lepidopterans (likely hawkmoths) to be playing a more significant role as pollinators as they visit the flowers earlier in the anthesis (Pimienta and Koptur, 2022) and are known to be more efficient pollen vectors than butterflies or skippers (Haber and Frankie, 1989; Johnson et al., 2017; Willmer, 2011). Their observed visits, however, were infrequent in comparison to the visits of diurnal Lepidoptera (Pimienta and Koptur, 2022), begging the question of which group of flower visitors are more important in the reproduction of *G. scabra*.

2. Methods

2.1 Study species— *Guettarda scabra* (Rubiaceae) is an evergreen shrub distributed throughout the Neotropics, from southern Florida in the US, the Caribbean region to northern Colombia and Venezuela (Acevedo-Rodriguez and Strong, 2012; Roberts, 2014; WCSP, 2022). In Florida, *G. scabra* is a pine rockland endemic, restricted to the southern portion of the peninsular pine rockland and hardwood hammock habitats, where it is relatively common (Tomlinson, 1980). Plants usually grow 2 – 3 m high but may reach 6 m in hammocks (Koptur and Garcia, 2017). Plants bloom during summer, from May through July in south Florida (Tomlinson, 1980). Flowers are grouped together on a long-stalked dichasial inflorescence (Tomlinson, 1980), with usually one to three sweetly fragrant flowers opening at time. They open in the late afternoon, stay open into the next morning, with most wilting by noon (Richards and Koptur, 1993). The corolla tube is white, usually flushed with pink, approximately 2 cm in length, and holds nectar at its base (Fig. 1A). Although *G. scabra* presents a special case of distyly, it is possible to identify the short-styled and long-styled morphs (Richards and Koptur, 1993). While *G. scabra* is capable of some self-fertilization, it depends on legitimate pollination for greater fruit production (Richards and Koptur, 1993). Fruits are drupes that expand and change from green to red color as they ripen from August to December (Fig. 1B) (Tomlinson, 1980; Koptur and Garcia, 2017).

2.2 Study site— We studied plants of *G. scabra* in south Florida (US) at Larry and Penny Thompson Memorial Park, Miami-Dade County (25°35′55″N 80°23′55″W). This park contains one of the largest fragments of pine rockland forest remaining in an urban area (Barrios et al., 2011) and is home to a large, natural population of *G. scabra*. Pine rocklands are restricted to south Florida and the Caribbean and are considered critically imperiled due to the extreme loss of their original area (Koptur, 2006; FNAI, 2010). This diminution is a result of many factors, all associated with human activities; outside of Everglades National Park only 2% of its original extent persists as small fragments (Snyder et al., 1990; Koptur, 2006; Possley et al., 2008; FNAI, 2010; Jones and Koptur, 2017). The pine rockland habitat is characterized by an understory of diverse herbaceous vegetation, a midstory comprised mainly of palms and shrubs, and a canopy dominated by the pine trees of *Pinus elliottii* (FNAI, 2010) (Fig. 1C). Over its entire extent, this habitat hosts over 552 plant species, of which 12% are endemic (Gann et al., 2002), including several rare and federally listed plants (FNAI, 2010).

2.3 Pollinator exclusion experiment— To evaluate the role of diurnal and nocturnal flower visitors in *G. scabra* female reproductive success, we carried out a pollinator exclusion experiment in an extensive natural population at Larry and Penny Thompson Memorial Park in Miami, Florida. During the flowering season of 2019, we selected 132 plants (68 short-styled and 64 long-styled morphs) with mature inflorescences, located at least 1 m apart from each other within the study area. We randomly assigned each plant to receive one of four pollinator exclusion treatments [33 plants (17 long-styled and 16 short-styled)/treatment, 2 inflorescences/plant]: a) nocturnal pollination, exclusion of diurnal visitors; b) diurnal pollination, exclusion of nocturnal visitors; c) closed to all pollinators and d) open to all pollinators (Fig. 2). The exclusion of diurnal pollinators was carried out from 0630 to1930 h, while the exclusion of nocturnal from 1930 to 0630 h. Treatments c and d served as negative and positive controls, respectively.

Pre-blooming inflorescences on each selected plant were tagged and their flower buds were counted before flower opening and fruit development. To restrict pollinator access, we enclosed the inflorescences with mesh bags made with 100% polyester fabric, and a mesh diameter of less than 1 mm, allowing adequate flow of air and light penetration (Fig. 2E). Each day at 0630 h, we bagged the inflorescences of each plant in the diurnal exclusion treatment and left exposed the ones in the nocturnal exclusion treatment. At 1930 h, we bagged the inflorescences of the individuals in the nocturnal exclusion treatment and uncovered the ones in the diurnal treatment. In all cases, we always kept the same bag for each inflorescence, pinning them to the stem to avoid inadvertent hand cross-pollination. Inflorescences subjected to the closed-to-pollinators treatment were always covered, whereas those under the open treatment were always exposed to floral visitors. We maintained these treatments for over a month (June 9 to July 13, 2019). When no more flowers were available on a studied inflorescence, we bagged it to protect and keep potential fruits. In November, after the fruits were fully developed, we checked and counted the fruits of inflorescences on all the experimental treatments. We used fruit-set (proportion of flowers setting fruit) as a proxy to evaluate the female reproductive success of individual plants.

2.4 Observation of flower visitors— To identify potential pollinators of *G. scabra* we observed flower visitors during the day and at night on plants with open flowers. Since most flowers open in the late afternoon and are wilted by noon, we made our observations during that time. Diurnal observations were conducted throughout the morning from 0730 to 1130 h (in 30 min periods), each day from 18 June to 8 July 2019. Nocturnal observations were performed during five non-consecutive days (2, 17 May and 2, 22, 26 June) from 1930 to 2330 h. For night observation we used headlamps with red-light filters to avoid disturbing the behavior of flower-visiting insects. Observations were carried out on a variable number of plants (ranging from 1 to 16) at time, subject to the availability of flowers. We registered visitation on each individual plant and noted the identity of visitors that made legitimate visits to flowers. We regard "legitimate" visits as those in which animals enter via the sympetalous corolla aperture, potentially contacting anthers and stigma. Visitors were identified visually and photographed or captured to confirm identification. Vouchers will be deposited in the Florida State Collection of Arthropods (Gainesville, FL, USA).

In total, we performed diurnal observations on 173 plants (0.5 h/plant), and nocturnal observations on 44 plants (4 h/plant). Visitation frequency of floral visitors was estimated by counting visits of legitimate visitors to the *G. scabra* plants per hour during the morning and at night (number of visits/plant/hour).

2.5 Statistical analyses— We evaluated both the differences in fruit set among the exclusion treatments and the visitation frequency among diurnal flower-visiting insects using a non-parametric ANOVA, Kruskal-Wallis test. When significant differences were found ($p < 0.05$), we used Dunn's test at $p = 0.05$ with Bonferroni correction for post-hoc comparisons between each pair of treatments. We assessed the differences between morning and night visitation frequencies using the Mann-Whitney test. All analyses were carried out using IBM SPSS Statistics, version 23.

3. Results

3.1 Pollinator exclusion experiment— We found that the mean fruit set of *G. scabra* plants differed significantly among individuals exposed to different pollinator exclusion treatments (Kruskal-Wallis, $\chi^2_3 = 36.718$, $P < 0.001$, N = 132 plants). Fruit production was significantly higher in both nocturnal (23.8%) and open (29.9%) treatments than in closed (4.2%) and diurnal (6.3%) treatments (Fig. 3). Post-hoc comparisons showed there was no difference in fruit set between plants under closed and diurnal treatments, as well as between those subjected to open and nocturnal treatments (Fig. 3).

3.2 Flower visitors— Flowers of *G. scabra* were visited legitimately by seven species of Lepidoptera in the morning and at night (Fig. 4). A total of 170 morning visits were performed by two species of butterflies (Nymphalidae): *Agraulis vanillae* and *Heliconius charithonia*; and four species of skippers (Hesperiidae): *Asbolis capucinus*, *Cymaenes tripunctus*, *Ephyriades brunnea* and *Polites baracoa*. Among them, *H. charithonia* was the main visitor (accounting for 57.1% of morning visits), followed by *P. baracoa* and *A. vanilla*, which were also common visitors (15.9% and 13.5% of the visits respectively), whereas the remaining diurnal lepidopterans were seen less frequently. In fact, *E. brunnea* was observed only once during the study whereas the other two skippers were seen more often (*A. capucinus* 8.2%, *C. tripunctus* 4.7%, *E.*

brunnea 0.6%). Butterflies and skippers tended to visit most of the available flowers on a single plant before moving on to the next plant.

At night, *G. scabra* flowers were visited only by *Xylophanes tersa* (Sphingidae). These hawkmoths were seen on only three occasions, during which they fed on flowers from two or three inflorescences per plant, on several plants, before flying away from the site.

3.3 Visitation frequency (No. of visits/plant/hour) — On average, individual plants received significantly more visits per hour during the morning (1.965) than at night (0.125) (U= 2790, P = 0.003, $N_{morning}$ = 173 plants and N_{night} = 44 plants) (Fig. 5).

Overall, the frequency of visitation differed among the diurnal species (Kruskal-Wallis, χ^2_5 = 118.038, P < 0.001) (Fig.6). Pairwise comparisons showed *H. charithonia* as the most frequent visitor (1.12 ± 2.01). The remaining species showed similar but lower visitation values (Fig. 6), except for *P. baracoa* (0.31± 1.06) and *E. brunnea* (0.01± 0.15) which differed in their visitation frequency (Fig. 6).

4. Discussion

Our exclusion experiment indicates that fruit-set in *G. scabra* results almost completely from nocturnal pollination, despite the significantly higher frequency of visits by diurnal lepidopterans. Dismissible fruit-set levels across treatments where nocturnal pollinators were excluded were not affected by the presence of diurnal visitors and hence are attributed to self-fertilization, as it is known to happen in *G. scabra* (Richards and Koptur 1993).

Our findings contrast with an earlier study (Koptur et al. 2021), in which an exclusion experiment reported a small but significant contribution to fruit set from diurnal visitors in *G. scabra*. Those investigators utilized a different experimental method, where all treatments were performed on each individual plant. It is possible that with the twice-daily visits to each plant there was a greater chance of disturbing the flowers and dislodging pollen from anthers to stigmas, boosting self-fertilization across all treatments equally. Such a disturbance would have had a relatively stronger effect on treatments with low fruit-set, like those where flowers were open to diurnal visitation only, making it difficult to evaluate any contribution by diurnal visitors. The association we found between *G. scabra* and nocturnal pollinators has also been suggested previously for other species in the genus *Guettarda*: *G. clarensis* (Martinez, 2013), *G. platypoda* (Darrault and Schlindwein, 2002, Novo et al., 2018), *G. viburnoides* (Loayza and Knight, 2010) and *G. speciosa* (Xu et al., 2018).

According to our results, nocturnal visitors act as effective pollinators, removing and transferring pollen among flowers on different individuals of *G. scabra*, thus being responsible for fruit production in this plant. Since the hawkmoth *X. tersa*, a species common in Florida during the blooming season for *G. scabra* (Kimball, 1965), was the only nocturnal visitor observed, we consider this hawkmoth as the most likely pollinator for *G. scabra* in the study area. We think that the relationship between *G. scabra* and *X. tersa* might be open on both sides, a “diffuse” coevolution (*sensu* Fox 1988). On the plant’s side, it is likely that other hawkmoths are present in the area and visit flowers of *G. scabra*. One such example could be *Perigonia lusca*, another species common in Miami Dade and whose caterpillars feed on *G. scabra*, among other Rubiaceae (Tuttle, 2007). *Perigonia lusca* was recently reported nectaring on flowers of *G. scabra* at night in the Everglades National Park (Pimienta and Koptur, 2022). Similarly, *Eumorpha fasciatus*, a hawkmoth considered very common and widespread in Florida (Kimball 1965), was also recently observed visiting flowers of *G. scabra* in south Florida (Pimienta and Koptur, 2022). However, we have no direct evidence that either of these species pollinate *G. scabra*, as they were not observed visiting flowers of this plant at this site during this study. On the hawkmoth's side, adults of *X. tersa* are generalist nectarivores (Tuttle, 2007), and have been observed feeding on flowers of *Lantana camara*, *Stachytarpheta* sp. (Verbenaceae), and *Mirabilis jalapa* (Nyctaginaceae) in suburban areas of south Florida (M.C.P. personal observations), as well as on *G. scabra* in the Everglades National Park (Pimienta and Koptur, 2022). Furthermore, populations of *X. tersa* are widely distributed well outside the areas where *G. scabra* can be found, ranging from Argentina (More et al., 2006) to southern Canada (d'Abrera, 1987), and have even been reported as a potential pollinator for *G. platypoda* (Novo et al., 2018). Despite the open nature of the plant-pollinator relationship in this system, the generalist habits of *X. tersa* might benefit small and relatively isolated populations of *G. scabra* concealed to suburban habitats where other pollinators cannot thrive.

Flower visits performed by the nocturnal hawkmoth *X. tersa* were much less frequent than those by diurnal Lepidoptera, which agrees with previous observations on hawkmoth visits in other night-blooming plants (e.g., Groman and Pellmyr, 1999; Reynolds et al., 2009; Kantsa et al., 2022). In fact, very low visitation rates by hawkmoths are common among sphingophilous plants (e.g., Oliveira and Gibbs, 2000; Wolf et al., 2003; Johnson et al., 2004; Oliveira et al., 2004), including another species in the genus *Guettarda*, *G. speciosa* (Xu et al., 2018). Low visitation rates by hawkmoth pollinators have been found even under long and intensive sampling conditions in southern Florida, where *Dendrophylax lindenii* (Orchidaceae) was visited by various hawkmoth species at irregular intervals and very low frequencies, over three years (Danaher et al., 2020).

In contrast with nocturnal visits where only *X. tersa* hawkmoths were observed, during the day flowers of *G. scabra* were frequented by several species of diurnal lepidopterans, in particular *Heliconius charithonia*, one of the few pollinivorous nymphalids (Gilbert, 1972; Jiggins, 2017; Young and Montgomery, 2020). The comparatively high frequency of flower visits by *H. charithonia* can be attributed to their natural history. *Heliconius* butterflies are avid floral visitors due to their dependence on pollen as a source of aminoacids, and also because its consumption enhances both their fertility and longevity (Gilbert, 1972; Boggs et al., 1981; Penz and Krenn 2000; Estrada and Jiggins, 2002). Furthermore, *Heliconius* species exhibit marked site-fidelity and homing capabilities (Gilbert, 1984; Mallet, 1986; Murawski and Gilbert, 1986; Moura et al., 2022), making them highly recurrent visitors to locally available pollen sources.

While a few species of *Heliconius* have specialized in visiting certain plant species (e.g., Murawski and Gilbert, 1986), *H. charithonia*, as most of the species in the genus, are generalist visitors that collect pollen based on the local availability of flowers (e.g., Cardoso, 2001; Estrada and Jiggins, 2002). High levels of visitation of *G. scabra* by local populations of *H. charithonia* demonstrate the reliance of these butterflies on floral rewards from this plant during summer. This may be because during this time *G. scabra* flowers are available while most of the other plants in the pine rockland are already in fruit (Loope, 1980; Gunderson et al., 1983).

In contrast with *H. charithonia*, diurnal lepidopterans feeding exclusively on nectar visited *G. scabra* much less frequently during this study. In the case of *Agraulis vanillae*, a nectar feeding nymphalid butterfly, infrequent flower visitation is likely a consequence of the ability of the caterpillars to accumulate lipid reserves and provide partial sustenance to the adult stage (May, 1992).

The remaining lepidopterans observed during the day were skippers (Hesperiidae) that have been previously reported visiting flowers of different plant species in pine rockland fragments. *Cymaenes tripunctus*, *Polites baracoa*, and *Asbolis capucinus* are opportunistic nectar-feeders known to visit other flowers in pine rockland fragments (Barrios et al., 2016; Koptur and Barrios, 2020). The presence of *A. capucinus* is likely linked to the high abundance of their larval host plant *Sabal palmetto* (Arecaeae) (Minno and Minno, 1999) in the study area. *Ephyriades brunnea* visited infrequently, as we also observed in the Everglades National Park (Pimienta and Koptur, 2022), this may be related to the local scarcity of the skipper's host plant *Byrsonima lucida* (Malpighiaceae) (Glassberg et al., 2000) and to a marked decline in populations of this skipper in Florida (Daniels, 2010; Reece et al., 2013).

The presence of diurnal visitors may suggest their potential role as pollinators. In fact, both diurnal and nocturnal visitors can be effective pollinators for some plants with predominantly nocturnal anthesis such as *Tillandsia heterophylla* (Bromeliaceae) (Aguilar-Rodriguez et al., 2016). Diurnal pollination can also play a role in plants across different families, where the main pollinators are nocturnal hawkmoths (e.g., Young, 2002; Oliveira et al., 2004; Walter, 2010; Doubleday and Eckert, 2018) and even in some sphingophilous Rubiaceae (e.g., Wolff et al., 2003; Oliveira et al., 2004; Maruyama et al., 2010). An interesting example is *Randia itatiaiae* (Rubiaceae) (de Avila and Freitas, 2011), a sphingophilous plant. Similar to *G. scabra*, *R. itatiaiae* is visited by both skippers and hawkmoths, with diurnal visits being much more frequent, though in that case both groups contribute equally to fruit set.

Although diurnal floral visitors have been reported to provide complementary services as pollinators in sphingophilous plants, we believe that this is not the case in *G. scabra*. Our findings suggest that diurnal lepidopterans visiting *G. scabra* act as “thieves” (Inouye, 1980) and “cheaters” (Soberón and Martínez del Río, 1985), consuming rewards from flowers without pollinating them, despite being legitimate visitors. *Heliconius* butterflies for example, visit flowers frequently and spend a relatively long time probing them (Penz and Krenn, 2000). However, it is unlikely that they transfer pollen among *G. scabra* flowers. This is because pollinivorous species, such as *H. charithonia,* remove pollen from flowers and keep it firmly attached to their mouthparts as it is being pre-digested (Jiggins, 2017). Plants that have co-evolved with heliconiines as their pollinators show a different set of traits than *G. scabra*. For example, species in the genus *Psiguria*, which are pollinated by *Heliconius* butterflies, produce large quantities of pollen in male flowers to fulfill the demands of the butterflies and still have enough for cross-pollination (Condon & Gilbert, 1990). Other nectar-feeding lepidopterans reported during this study may also be ineffective pollen vectors for *G. scabra*. All of them, with the exception of *E. brunnea,* were previously observed visiting frequently *Angadenia berteroi*

(Apocynaceae) in pine rockland habitats of south Florida, yet none removed or transferred pollen during visitation (Barrios et al., 2016).

Our findings suggest that *G. scabra* has a rather specialized pollination system, where hawkmoths are the only group responsible for the reproductive success of the plant. The evidence presented here further supports the nocturnal moth pollination syndrome in *G. scabra*, in which floral traits such as white and long corolla tube, anthesis occurring at late evening, and the emission of a strong sweet scent suggest that this plant is adapted to night-active visitors. Such specialization is consistent with known associations between hawkmoth pollinators and other species in the genus *Guettarda*. For example, *Guettarda speciosa* is only pollinated by the hawkmoth *Cephonodes hylas*, despite their very low frequency of floral visits and the presence of diurnal visitors, such as bees and flies that were acting as nectar thieves (Xu et al., 2018). Similarly, a clear association between *G. platypoda* and *X. tersa* has been established by direct observation of floral visits (Novo et al., 2018) and pollen loads on hawkmoths (Darrault and Schlindwein, 2002). Additional associations with night-active hawkmoths have been also suggested for *G. clarensis* (Martinez, 2013) and *G. viburnoides* (Loayza and Knight, 2010).

Conclusions

The rough leaf velvet seed *G. scabra* is one of the main components of the understory of the pine rockland habitat. While anthesis in this species is predominantly nocturnal, their flowers are mainly visited by several species of diurnal lepidopterans. Regardless of intense diurnal visitation and a residual capability for self-fertilization, our findings support the idea that *G. scabra* has a specialized pollination system. In this system, *X. tersa* and likely other nocturnal hawkmoths are the only effective pollinators, while diurnal visitors behave as nectar and pollen thieves.

Since plants exhibiting highly specialized pollination systems are associated with particular pollen vectors, the disruption of pollinator services may strongly affect their sexual reproduction. In hawkmoth-pollinated plants, a reduction in abundance and diversity of hawkmoths might threaten their reproductive success by limiting pollen transfer (Johnson et al., 2004, Amorim et al., 2014). Furthermore, hawkmoth-pollinated plants surviving only in remnant habitats may be especially at risk due to the detrimental effects of fragmentation on pollination services (Johnson et al., 2004). In *G. scabra*, the dependence on a single and infrequent hawkmoth species to set fruits may endanger the continuity of local populations, particularly those confined to suburban pine rockland fragments in south Florida.

The continued existence of *G. scabra* relies strongly on the presence of hawkmoths. Diversity and abundance of hawkmoths is reduced in proximity to human habitation as the use of insecticides and paucity of hostplants can limit their survival. In gardening for pollinators, we must keep in mind the needs of these important nocturnal Lepidoptera, highly effective pollen transporters that need not only nectar resources but their host plants as well.

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Author contributions

María Cleopatra Pimienta: Conceptualization, methodology, funding acquisition, investigation, data collection, curation, and analysis, writing - original draft. **Carlos Ruiz**: Data collection and analysis, writing – original draft and editing. **Suzanne Koptur**: Conceptualization, methodology, supervision, writing - reviewing and editing.

Data availability statement

The data that support the findings of this study will be openly available in the FIU Research Data Portal at https://doi.org/10.34703/gzx1-9v95/CLZMLA

Conflict of interest statement

The authors have no conflict of interest to declare

References

Acevedo-Rodríguez, P., and Strong, M. T. 2012. Catalogue of seed plants of the West Indies. *Smithsonian Contributions to Botany*, 98, 1– 1192. https://doi.org/10.5479/si.0081024X.98.1

Aguilar-Rodríguez, P. A., Krömer, T., García-Franco, J. G., and MacSwiney, G. M. C. 2016. From dusk till dawn: nocturnal and diurnal pollination in the epiphyte

Tillandsia heterophylla (Bromeliaceae). *Plant Biology*, 18(1), 37–45. https://doi.org/ 10.1111/plb.12319

Amorim, F. W., Wyatt, G. E., and Sazima, M. 2014. Low abundance of long-tongued pollinators leads to pollen limitation in four specialized hawkmoth-pollinated plants in the Atlantic Rain forest, Brazil. *Naturwissenschaften*, 101(11), 893–905. https://doi.org/ 10.1007/s00114-014-1230-y

Baker, H. G., and Hurd Jr., P. D. 1968. Intrafloral ecology. *Annual Review of Entomology*, 13, 385–414. https://doi.org/10.1146/annurev.en.13.010168.002125

Barrios, B., Arellano, G., and Koptur, S. 2011. The effects of fire and fragmentation on occurrence and flowering of a rare perennial plant. *Plant Ecology*, 212, 1057–1067. https://doi.org/10.1007/s11258-010-9886-7

Barrios, B., Pena, S. R., Salas, A., and Koptur, S. 2016. Butterflies visit more frequently, but bees are better pollinators: the importance of mouthpart dimensions in effective pollen removal and deposition. *AoB Plants,* 8, plw001. https://doi:10.1093/aobpla/plw001

Boggs, C. L., Smiley, J.T., Gilbert, L. E. 1981. Patterns of pollen exploitation by *Heliconius* butterflies. *Oecologia*, 48,284–89. https://doi.org/10.1007/BF00347978

Cardoso, M. Z. 2001. Patterns of pollen collection and flower visitation by *Heliconius* butterflies in southeastern Mexico. *Journal of Tropical Ecology*, 17(5), 763–768. https://doi.org/10.1017/S0266467401001572

Castro, S., Loureiro, J., Ferrero, V., Silveira, P., and Navarro, L. 2013. So many visitors and so few pollinators: variation in insect frequency and effectiveness governs the reproductive success of an endemic milkwort. *Plant Ecology*, 214(10), 1233–1245. https://doi.org/10.1007/s11258-013-0247-1

Condon, M.A, and Gilbert, L. E. 1990. Reproductive biology and natural history of the Neotropical vines *Gurania* and *Psiguria*. In: Bates D., Robinson R. W., Jeffrey C. (eds.) Biology and utilization of the Cucurbitaceae, 150–166. Cornell University Press, Ithaca, New York.

d'Abrera, B. 1987. Sphingidae Mundi: hawk moths of the world: based on a checklist by Alan Hayes and the collection he curated in the British Museum (Natural History). 226 pp. E. W. Classey Ltd., Faringdon, Oxon, UK.

Danaher, M. W., Ward, C., Zettler, L.W., and Covell, C.V. 2020. Pollinia removal and suspected pollination of the endangered ghost orchid, *Dendrophylax lindenii* (Orchidaceae) by various hawk moths (Lepidoptera: Sphingidae): Another mystery dispelled. *Florida Entomologist*, 102(4), 671–683. https://doi.org/10.1653/024.102.0401

Daniels, J. 2010. Florida butterflies. *Wings: Essays on Invertebrate Conservation*, 33(2), 18–21. Retrieved from https://xerces.org/wings

Darrault, R. O., and Schlindwein, C. 2002. Esfingídeos (Lepidoptera, Sphingidae) no Tabuleiro Paraibano, nordeste do Brasil: abundância, riqueza e relação com plantas esfingófilas. *Revista Brasileira de Zoologia*, 19, 429–443. https://doi.org/10.1590/S0101-81752002000200009

de Avila, R. S., and Freitas, L. 2011. Frequency of visits and efficiency of pollination by diurnal and nocturnal lepidopterans for the dioecious tree *Randia itatiaiae* (Rubiaceae). *Australian Journal of Botany*, 59(2), 176–184. https://doi.org/10.1071/BT10280

de Merxem, D. G., Borremans, B., De Jäger, M. L., Johnson, T., Jooste, M., Ros, P., Zenni, R. D., Ellis, A. G. and Anderson, B. 2009. The importance of flower visitors not predicted by floral syndromes. *South African Journal of Botany*, 75(4), 660–667. https://doi.org/10.1016/j.sajb.2009.08.002

Doubleday, L. A., and Eckert, C. G. 2018. Experimental evidence for predominant nocturnal pollination despite more frequent diurnal visitation in *Abronia umbellata* (Nyctaginaceae). *Journal of Pollination Ecology*, 22, 67–74. https://doi.org/10.26786/1920-7603(2018)six

Estrada, C., and Jiggins, C. D. 2002. Patterns of pollen feeding and habitat preference among *Heliconius* species. *Ecological Entomology*, 27(4), 448–56. https://doi.org/10.1046/j.1365-2311.2002.00434.x

Faegri, K., and van der Pilj, L. 1979. *The Principles of Pollination Ecology*. Oxford, UK: Pergamon Press.

Fishbein, M., and Venable, D. L. 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology*, 77(4), 1061–1073. http://doi.org/10.2307/2265576

Florida Natural Areas Inventory (FNAI). 2010. Pine Rockland. In: Guide to the natural communities of Florida, 69–72. Florida Natural Areas Inventory, Tallahassee, FL.

Fox, L .R. 1988. Diffuse coevolution within complex communities. *Ecology,* 69(4), 906-907. https://doi.org/10.2307/1941243

Gann, G. D., Bradley, K. A., and Woodmansee, S. W. 2002. Rare plants of South Florida: Their history, conservation, and restoration. The Institute for Regional Conservation, Miami, Florida, U.S.A

Gilbert, L. E. 1972. Pollen feeding and reproductive biology of *Heliconius* butterflies. *Proceedings of the National Academy of Sciences*, 69(6), 1403–1407. https://doi.org/10.1073/pnas.69.6.1403

Gilbert, L. E. 1984. The biology of butterfly communities. In: Vane-Wright R. I. and Ackery P. R. (eds.) The biology of butterflies, 41–54. Academic Press, London.

Glassberg, J., Minno, M. C., and Calhoun, J. V. 2000. Butterflies through binoculars: A field, finding, and gardening guide to butterflies in Florida. Oxford University Press, New York.

Gribel, R., and Hay, J. 1993. Pollination ecology of *Caryocar 72rasiliense* (Caryocaraceae) in Central Brazil cerrado vegetation. *Journal of Tropical Ecology*, 9(2), 199–211. https://doi:10.1017/S0266467400007173

Groman, J. D., and Pellmyr, O., 1999. The pollination biology of *Manfreda virginica* (Agavaceae): relative contribution of diurnal and nocturnal visitors. *Oikos,* (87), 373–381. https://doi.org/10.2307/3546753

Gunderson, L. H., Taylor, D. L., and Craig, J. 1983. Fire effects on flowering and fruiting patterns of understory plants in pinelands of Everglades National Park; National Park Service, Report SFRC-83/04; South Florida Research Center, Homestead, FL, 36p.

Haber, W. A., and Frankie, G. W. 1989. A tropical hawkmoth community: Costa Rican dry forest Sphingidae. *Biotropica*, 21, 155–172. https://doi.org/10.2307/2388706

Inouye, D. W. 1980. The terminology of floral larceny. *Ecology*, 61, 1251–1253. https://doi.org/10.2307/1936841

Jiggins, C. D. 2017. The ecology and evolution of *Heliconius* butterflies. Oxford University Press. United Kingdom.

Johnson, S. D., Neal, P. R., Peter, C. I., and Edwards, T. J. 2004. Fruiting failure and limited recruitment in remnant populations of the hawkmoth-pollinated tree *Oxyanthus pyriformis* subsp. *pyriformis* (Rubiaceae). *Biological Conservation,* 120(1), 31–39. https://doi.org/10.1016/j.biocon.2004.01.028

Johnson, S. D., Moré, M., Amorim, F. W., Haber, W. A., Frankie, G.W., Stanley, D. A., Cocucci, A. A., and Raguso, R. A. 2017. The long and the short of it: a global analysis of hawkmoth pollination niches and interaction networks. *Functional Ecology,* 31, 101–115. https://doi.org/10.1111/1365-2435.12753

Jones, I. M., and Koptur, S. 2017. Dead land walking: the value of continued conservation efforts in South Florida's imperiled pine rocklands. *Biodiversity and Conservation*, 26(14), 3241–3253. https://doi.org/10.1007/s10531-017-1433-6

Kantsa, A., Garcia, J. E., Raguso, R. A., Dyer, A .G., Steen, R., Tscheulin, T., and Petanidou, T., 2022. Intrafloral patterns of color and scent in *Capparis spinosa* L. and the ghosts of its selection past. *American Journal of Botany*, e16098. https://doi.org/10.1002/ajb2.16098

Kimball, C. P., 1965. The Lepidoptera of Florida: an annotated checklist (Vol. 1). Division of Plant Industry, State of Florida Department of Agriculture, Gainesville, FL. 363 pp.

Koptur, S. 2006. The conservation of specialized and generalized pollination systems in subtropical ecosystems: a case study. In: Waser N., and Ollerton J. (eds.) Plant–pollinator interactions: from specialization to generalization, 341–361. University of Chicago Press, Chicago.

Koptur, S. 2020. Scientific Note: Insects associated with *Guettarda scabra* in Everglades National Park, Florida. *Castanea*, 85(1), 155–158. https://doi.org/10.2179/0008-7475.85.1.155

Koptur, S., and Barrios, B. 2020. Are native palms "pollinator hogs"? A field experiment in pine rocklands of southern Florida. *Natural Areas Journal*, 40(2),142–149. https://doi.org/10.3375/043.040.0205

Koptur, S., and Garcia, D. 2017. Habitat differences in morphology and reproductive allocation in *Guettarda scabra* (Rubiaceae). *Castanea*, 82(2), 51–57. https://doi.org/10.2179/16-115

Koptur, S., Peña, S., and Barrios, B. 2021. Do Morning Butterfly Visitors Benefit a Night-Flowering Hawkmoth Pollinated Plant? *Castanea*, 86(1), 100–111. https://doi.org/10.2179/0008-7475.86.1.100

Loayza, A. P., and Knight, T., 2010. Seed dispersal by pulp consumers, not "legitimate" seed dispersers, increases *Guettarda viburnoides* population growth. *Ecology*, 91(9), 2684–2695. https://doi.org/10.1890/09-0480.1

Loope, L. L. 1980. Phenology of flowering and fruiting in plant communities of Everglades National Park and Biscayne National Monument, Florida; Report T-593; South Florida Research Center, Homestead, FL, 50pp.

Soberón, J., and C. Martínez del Río. 1985. Cheating and taking advantage in mutualistic associations. In: Boucher D. (ed.) The biology of mutualism: ecology and evolution, 192–216. Oxford University Press, New York.

Mallet, J. 1986. Dispersal and gene flow in a butterfly with home range behavior: *Heliconius erato* (Lepidoptera: Nymphalidae). *Oecologia*, 68(2), 210–217. https://doi.org/10.1007/BF00384789

Martínez, L. 2013. Fenología reproductiva y efecto del robo de néctar en el éxito reproductivo de *Guettarda clarensis*, en. Bachelor's Thesis, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Villa Clara, Cuba, 2013. Available online: https://dspace.uclv.edu.cu/handle/123456789/1723 (accessed on 19 October 2022).

Martins, C., Oliveira, R., Mendonca, C.V., Lopes, L. T., Silveira, R. A., de Silva, J. A. P., Aguiar, L. M. S., and Antonini, Y. 2016. Reproductive biology of *Cipocereus minensis* (Cactaceae) — A columnar cactus endemic to rupestrian fields of a Neotropical savannah. *Flora*, 218, 62–67. https://doi.org/10.1016/j.flora.2015.11.010

Maruyama, P. K., Amorim, F.W., and Oliveira, P. E. 2010. Night and day service: distyly and mixed pollination system in *Faramea cyanea* (Rubiaceae). *Flora*, 205 (12), 818–824. https://doi.org/10.1016/j.flora.2010.01.004

May, P. G. 1992. Flower selection and the dynamics of lipid reserve in two nectarivorous butterflies. *Ecology*, 73(6), 2181–2191. https://doi.org/10.2307/1941466

Minno, M. C., and Minno, M. 1999. Florida butterfly gardening: A complete guide to attracting, identifying, and enjoying butterflies. University Press of Florida, Gainesville.

Miyake, T., and Yahara, T. 1998. Why does the flower of *Lonicera japonica* open at dusk? *Canadian Journal of Botany* 76(10), 1806–1811. http://doi.org/10.1139/b98-119

More, M., Sersic, A. N., and Cocucci, A. A. 2006. Specialized use of pollen vectors by *Caesalpinia gilliesii*, a legume species with brush-type flowers. *Biological Journal of the Linnean Society*, 88(4), 579–592. https://doi.org/10.1111/j.1095-8312.2006.00644.x

Moura, P. A., Corso, G., Montgomery, S. H., and Cardoso, M. Z. 2022. True site fidelity in pollen-feeding butterflies. *Functional Ecology*, 36(3), 572–582. http://doi.org/10.1111/1365-2435.13976

Murawski, D. A., and Gilbert, L. E. 1986. Pollen flow in *Psiguria warscewiczii*: a comparison of *Heliconius* butterflies and hummingbirds. *Oecologia*, *68*(2), 161–167. http://doi.org/10.1007/bf00384782

Novo, R. R., Consolaro, H., Almeida, N. M., and Castro, C. C. 2018. Floral biology of the velvetseed *Guettarda platypoda* DC. (Rubiaceae): Atypical distyly or style dimorphism? *Flora*, 239: 62–70. https://doi.org/10.1016/j.flora.2017.11.008

Oliveira, P. E., and Gibbs, P. E. 2000. Reproductive biology of woody plants in a Cerrado community of Central Brazil. *Flora*, *195*(4), 311–329. https://doi.org/10.1016/S0367-2530(17)30990-8

Oliveira, P. E., Gibbs, P. E., and Barbosa, A. A. 2004. Moth pollination of woody species in the Cerrados of Central Brazil: a case of so much owed to so few? *Plant Systematics and Evolution*, *245*(1), 41–54. https://doi.org/10.1007/s00606-003-0120-0

Penz, C. M., and Krenn, H. W. 2000. Behavioral adaptations to pollen-feeding in *Heliconius* butterflies (Nymphalidae, Heliconiinae): an experiment using *Lantana* flowers. *Journal of Insect Behavior*, 13(6), 865–880. http://doi.org/10.1023/A:1007814618149

Pimienta, M. C., and Koptur, S. 2022. More than moths: Flower visitors of a night-blooming plant in south Florida pine rocklands, USA. *Plants*, 11, 2799. https://doi.org/10.3390/plants11202799

Possley, J., Woodmansee, S. W., and Maschinski, J. 2008. Patterns of plant composition in fragments of globally imperiled pine rockland forest: Effects of soil type, recent fire frequency, and fragment size. *Natural Areas Journal*, 28, 379–394. https://doi.org/10.3375/0885-8608(2008)28[379:POPCIF]2.0.CO;2

Reece, J. S., Noss, R. F., Oetting, J., Hoctor, T., and Volk, M. 2013. A vulnerability assessment of 300 species in Florida: threats from sea level rise, land use, and climate change. *PloS One*, 8(11), 80658. https://doi.org/10.1371/journal.pone.0080658

Reynolds, R. J., Westbrook, M .J., Rohde, A. S., Cridland, J. M., Fenster, C. B., and Dudash, M. R. 2009. Pollinator specialization and pollination syndromes of three related North American *Silene*. *Ecology*, 90(8), 2077–2087. https://doi.org/10.1890/08-1141.1

Richards, J. H., and Koptur, S. 1993. Floral variation and distyly in *Guettarda scabra* (Rubiaceae). *American Journal of Botany*, 80(1), 31–40. https://doi.org/10.1002/j.1537-2197.1993.tb13764.x

Roberts, A. 2014. *Guettarda scabra*. The IUCN Red List of Threatened Species 2014: e.T56503696A56503850. https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T56503696A56503850.en.

Snyder, J. R., Herndon, A., and Robertson, Jr., W. B. 1990. South Florida rockland. In: Myers, R. L., and Ewel, J. J. (eds.) Ecosystems of Florida, 230–274. University of Central Florida Press, Orlando, FL.

Tomlinson, P. B. 1980. The biology of trees native to tropical Florida. Harvard University Printing Office. Allston, MA.

Tuttle, J. P. 2007. The hawk moths of North America: a natural history study of the Sphingidae of the United States and Canada; Wedge Entomological Research Foundation: Washington, DC.

Walter, H. E. 2010. Floral biology *of Echinopsis chiloensis* ssp. *chiloensis* (Cactaceae): evidence for a mixed pollination syndrome. *Flora*, 205(11), 757–763. https://doi.org/10.1016/j.flora.2009.12.038

WCSP, 2022. World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew. Retrieved from http://wcsp.science.kew.org

Willmer, P. 2011. Pollination and floral ecology. Princeton University Press, Princeton, New Jersey.

Wolff, D., Braun, M., and Liede, S. 2003. Nocturnal versus diurnal pollination success in *Isertia laevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds. *Plant Biology*, 5(1), 71–78. http://doi.org/10.1055/s-2003-37977

Wolff, D., Witt, T., Jürgens, A., and Gottsberger, G. 2006. Nectar dynamics and reproductive success in *Saponaria officinalis* (Caryophyllaceae) in southern Germany. *Flora*, 201(5), 353–364. https://doi.org/10.1016/j.flora.2005.07.010

Xu, Y., Luo, Z., Gao, S., and Zhang, D. 2018. Pollination niche availability facilitates colonization of *Guettarda speciosa* with heteromorphic self-incompatibility on oceanic islands. *Scientific Reports*, 8(1), 1–12. https://doi.org/10.1038/s41598-018-32143-5.

Young, F. J., and Montgomery, S. H. 2020. Pollen feeding in *Heliconius* butterflies: The singular evolution of an adaptive suite. *Proceedings of the Royal Society*, B *287*(1938), 20201304. http://dx.doi.org/10.1098/rspb.2020.1304

Young, H. J. 2002. Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). *American Journal of Botany*, 89(3), 433–440. http://doi.org/10.3732/ajb.89.3.433

Figures

Figure 1. *Guettarda scabra* flowers, fruits, and habitat. (A) Open flowers on two parts of the dichasial cyme. (B) Immature and mature fruit on inflorescences subtended by tough, scabrous leaves. (C) General view of pine rockland habitat at Larry and Penny Thompson Memorial Park, Miami-Dade County, Florida, US.

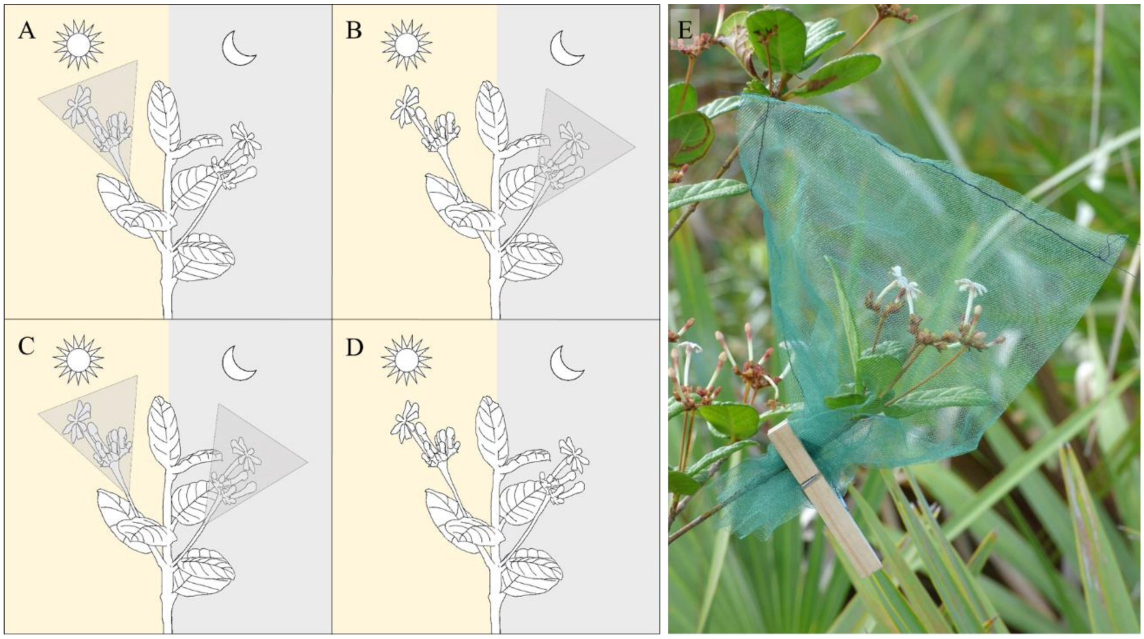

Figure 2. Pollinator exclusion experiment performed on *Guettarda scabra* plants. Each selected plant (2 inflorescences per plant) received one out four treatments: (A) exclusion of diurnal visitors, (B) exclusion of nocturnal visitors, (C) exclusion of all visitors, and (D) open to all visitors. Diurnal visitors were excluded from 0630 to1930 h, and the nocturnal from 1930 to 0630 h. Treatments C and D were negative and positive controls respectively. (E) A nylon bag used to prevent visitation covered inflorescences

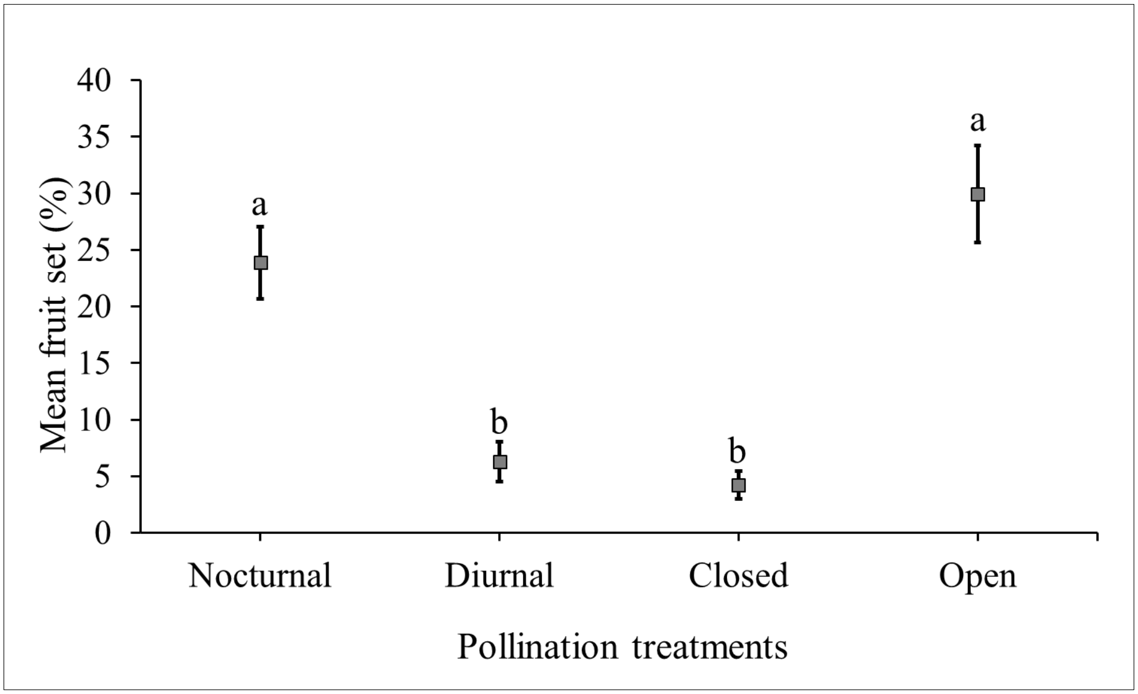

Figure. 3. Average percent fruit set (mean ± SE) of *Guettarda scabra* plants as a result of four treatments (nocturnal: exclusion of diurnal visitors, diurnal: exclusion of nocturnal visitors, closed: exclusion of all visitors, and open: exposed to all visitors); 33 plants per treatment, 2 inflorescences per plant. Kruskal-Wallis test, X^2_3=36.71, P<0.001, significant differences (p <0.05) between treatments by the Dunn's test with Bonferroni correction are indicated by different letters above bars.

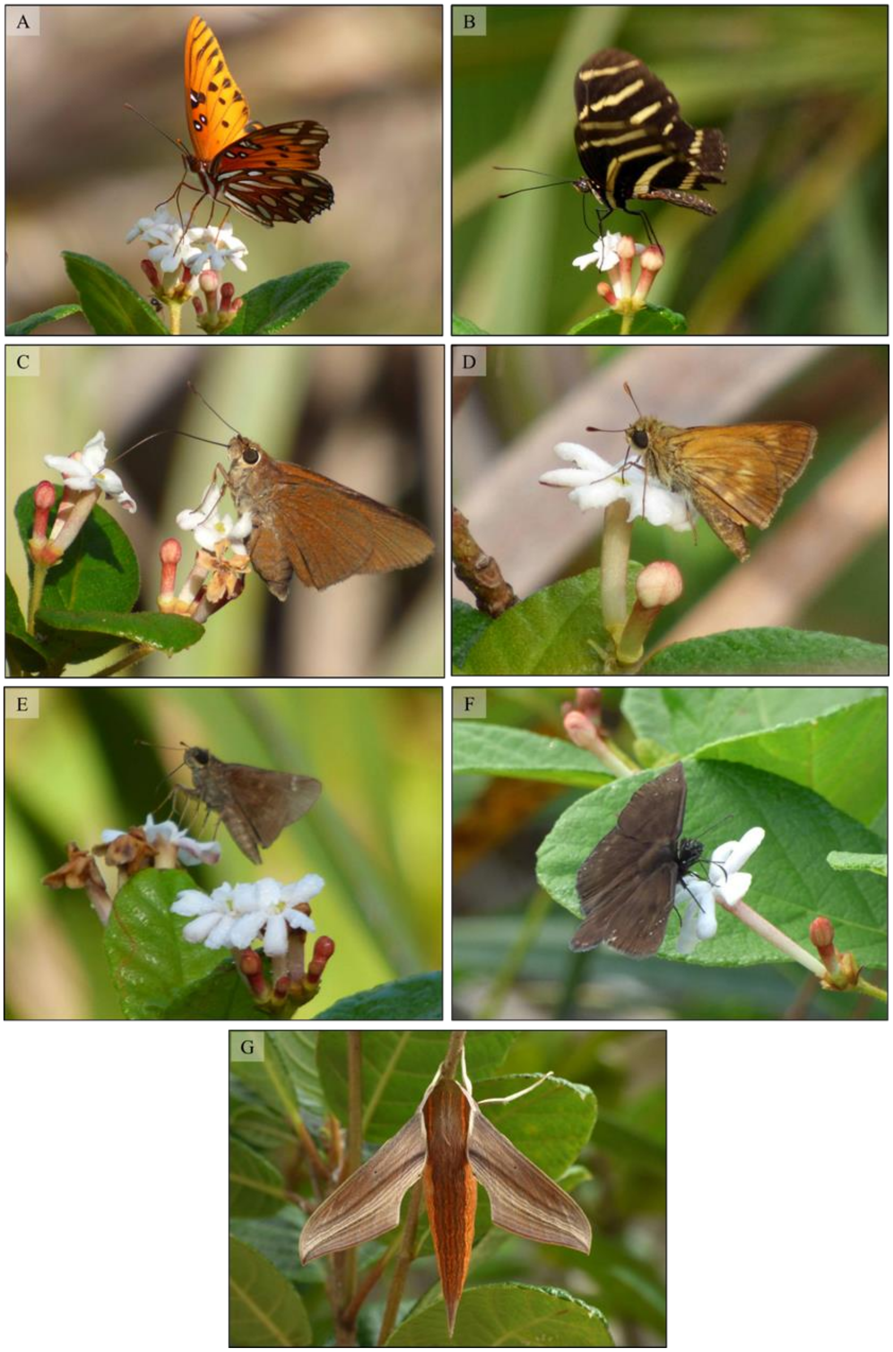

Figure 4. Legitimate flower visitors of *Guettarda scabra* (Rubiaceae) recorded during the flowering season of 2019 in a pine rockland forest, Larry and Penny Thompson Memorial Park, south Florida, USA. Diurnal visitation by butterflies/Nymphalidae: (A) *Agraulis vanillae* Linnaeus, (B) *Heliconius charithonia* Linnaeus; and skipper butterflies /Hesperiidae: (C) *Asbolis capucinus* Lucas, (D) *Polites baracoa* Lucas, (E) *Cymaenes tripunctus* Herrich-Schäffer, (F) *Ephyriades brunnea* Herrich-Schäffer; (G) hawkmoth/ Sphingidae: *Xylophanes tersa* Linnaeus, night-active visitor, and the only nocturnal pollinator observed.

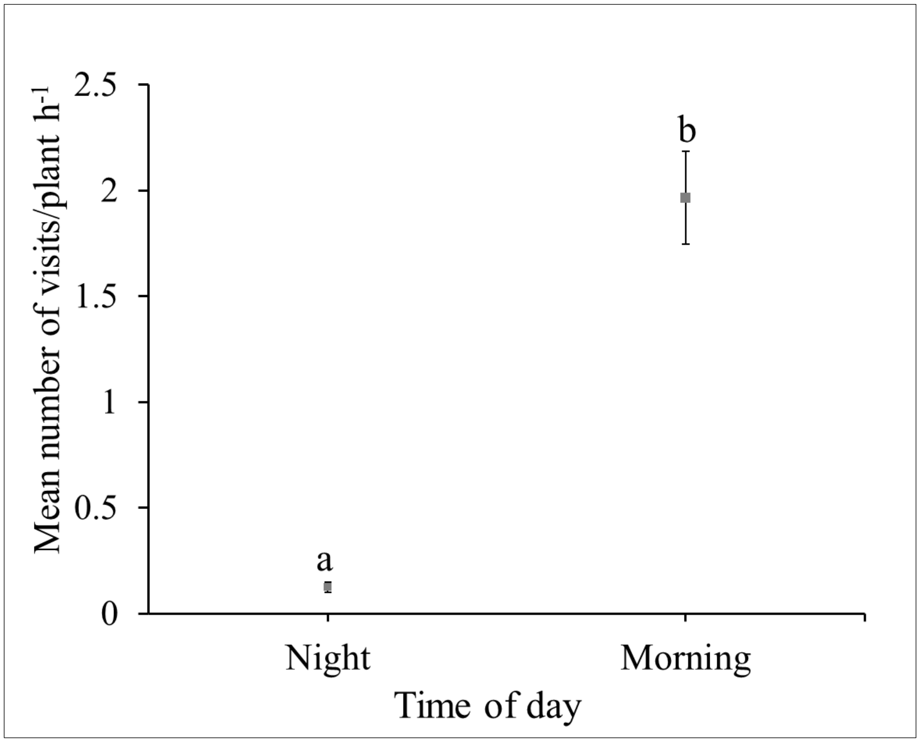

Figure 5. Visitation frequency (No. of visits/plant/hour). Mean (± SE) number of visits received by *Guettarda scabra* plants per hour during the morning and at night. Mann-Whitney test, U= 2790, P = 0.003, $N_{morning} = 173$ and $N_{night} = 44$. Different superscript letters indicate significant differences at $p < 0.05$. N is the total number of plants observed through the study during the morning and at night.

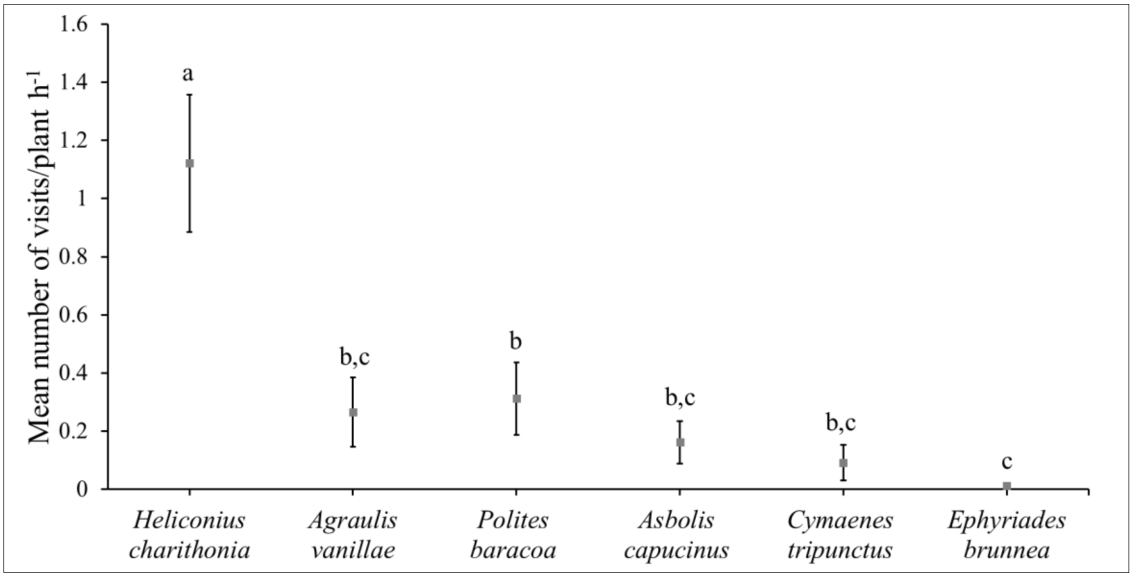

Figure 6. Visitation frequency (No. of visits/plant/hour) of the diurnal visitors. Mean (± SE) number of visits of different flower visitors species received per *Guettarda scabra* plants per hour. Kruskal-Wallis test, X^2_5=118.038, P<0.001. Significant differences (p <0.05) between visitors by Dunn's test with Bonferroni correction are indicated by different letters above bars.

CHAPTER IV

THE NIGHTTIME FRAGRANCE OF *GUETTARDA SCABRA* (RUBIACEAE): FLOWER SCENT AND ITS IMPLICATIONS FOR MOTH POLLINATION.

Abstract

Floral scent plays a crucial role in attracting pollinators, particularly plants that bloom at night. Despite their significance, chemical profiles of flowers from nocturnal distylous plants are poorly documented, limiting our understanding of their pollination ecology. I investigated the floral scent in *Guettarda scabra* (L.) Vent. (Rubiaceae), a night-blooming species with short- and long-styled floral morphs, endemic to south Florida's threatened pine rockland and hardwood hammock habitats. To obtain chemical profiles for the floral scent of both morphs, I used dynamic headspace sampling and GC-MS analysis. Additionally, I used neutral red staining to identify the specific floral regions responsible for scent emission in the flowers of this species. My findings show that the fragrance of *G. scabra* flowers consists entirely of benzenoid and terpenoid compounds and is characterized by the dominance of benzeneacetaldehyde and (E)-ß-ocimene. Furthermore, I found no difference between the chemical profiles of long- and short styled flowers. Finally, staining assays revealed that the primary sources of the flower's scent in *G. scabra* are the corolla lobes, and likely also the anthers and stigma. These results indicate that *G. scabra*'s floral scent profile is consistent with that of night-blooming plants pollinated by nocturnal hawkmoths. Based on its composition, floral scent in *G. scabra* may also mediate interactions with other diurnal and nocturnal flower-visiting insects. This work constitutes the first study of the chemical composition of floral scent in a species in the genus *Guettarda*, providing a baseline for understanding the chemical ecology of pollinator attraction to *G. scabra*. This study shows a case where the floral traits, including floral volatiles, support the association between a plant and its effective pollinator. By

comprehending the chemical ecology of the floral scent, we can better understand how a plant attracts its pollinators, and potentially develop strategies toward their conservation.

Keywords

Florida; flower scent; GC-MS; night-blooming plant; pine rockland; scent localization; volatile organic compounds, VOCs.

Introduction

Floral scent is one of the key traits that plants use to achieve successful reproduction because it contributes to attracting pollen vectors and thus promote cross-pollination (Raguso, 2008; Willmer, 2011). The emission of floral scent is particularly important for night-blooming plants that depend on flower visitors for pollen transfer (Borges et al., 2016). At night, visual cues become less effective over long distances, making chemical cues essential in attracting pollinators close enough, where both senses can be used to determine the exact location of a flower (Dobson, 1994; Borges et al., 2016).

Scent emission usually happens right before anthesis and signals the availability of floral rewards to nearby pollen vectors (Dudareva et al., 1999). Fragrance release usually peaks when the flower is receptive for pollination (Duradera and Pichersky, 2006), and frequently correlates with the peak activity of moths in sphingophilous plants (Effmert et al., 2005; Okamoto et al., 2008; Powers et al., 2020). In general, floral fragrances are mixtures of volatile organic compounds (VOCs) produced by floral tissues, particularly petals (Dobson et al., 2005). These mixtures can contain a diverse array of chemical groups such as aliphatics, benzenoids, terpenoids, as well as nitrogen- and sulphur-containing compounds (Dobson et al., 2005; Knudsen et al., 2006; Willmer, 2011). Most flowers produce complex odor blends that typically contain 20–60 chemical compounds. (Knudsen et al. 1993, Knudsen and Gershenzon, 2006; Willmer, 2011). The richness and diversity of these compounds vary widely interspecifically (Knudsen and Gershenzon, 2006), and can even differ between floral morphs within a species (Johnson et al., 2019).

Flowers of different plants are characterized by specific ratios of volatiles compounds, often resulting in a unique fragrance that can attract certain guilds of pollinators or even particular species (Dobson, 2006; Willmer 2011). In particular, nocturnal flowers that are pollinated by sphingid moths have a distinctive strong and scent, which is usually dominated by terpenoids and benzenoids, and also may include nitrogen-containing compounds (Knudsen and Tollsten, 1993; Miyake et al., 1998; Dobson, 2006). Besides the chemical composition, and along with other sensory information, the spatial distribution and concentration of floral scent results in the selective attraction of pollinators to a flower (Raguso, 2008). This selective targeting is compounded by the fact that most insect pollinators learn to associate a particular floral bouquet to a specific food reward (Cunningham et al., 2004; Dobson et al., 2005; Riffell, 2011; Willmer, 2011), thanks to their remarkable cognitive flexibility (Kelber, 2002; Riffell et al., 2008; Jones and Agrawal, 2017), and also to link the maximum emission of particular floral VOCs to the availability of high-quality rewards (Dobson et al. 2005; Wright and Schiestl, 2009). These associations enhance flower fidelity in many insects, thereby increasing their efficiency as pollen vectors (Dobson et al. 2005; Wright and Schiestl, 2009). Such mutualistic relationship has promoted coevolutionary dynamics across multiple times, resulting in plants targeting highly effective pollinators by including, in their floral scent, compounds that act as strong attractants to that group (Dobson, 2006).

Regardless of the relevance of floral scents for nocturnal distylous plants, they are poorly documented, limiting our understanding of their pollination ecology. A clear understanding of pollination ecology is particularly important to design and implement conservation strategies for native plants whose habitats are being decimated by aggressive fragmentation. This is the case of the rough-leaf velvetseed *Guettarda scabra* (L.) Vent. (Rubiaceae), a night-blooming species that is native to south Florida's threatened pine rockland and hardwood hammock habitats in the US (Tomlinson, 1980). These habitats have lost most of its original coverage over the last century and are considered imperiled due to severe anthropogenic fragmentation (Snyder et al., 1990; Koptur, 2006; Possley et al., 2008; FNAI, 2010; Jones and Koptur, 2017).

Guettarda scabra is a night-blooming plant, considered morphologically distylous, with short- and long-styled floral morphs (Figure 1). Flowers in this species are characterized by a white and long tubular corolla, nectar secretion, and by the emission of a strong fragrance at night (Figure 1). This combination of traits has long suggested that *G. scabra* is pollinated by nocturnal moths, particularly hawkmoths (Sphingidae) (Faegri and van der Pijl, 1979; Willmer, 2011), an association frequently observed in species in the Rubiaceae family (Bawa et al., 1985; Haber and Frankie, 1989). In accordance with these predictions, the association between *G. scabra* and nocturnal hawkmoth pollinators was recently confirmed (Pimienta et al., submitted).

Despite being an important source of floral rewards for the local arthropod fauna (Pimienta and Koptur, 2022), many basic aspects of the floral ecology of *G. scabra* remain unknown, such as those related to floral scent emission and composition. To address these gaps, I set out to determine the chemical profile of the floral scent in *G. scabra* and evaluate whether this profile is conserved across floral morphs. Additionally, I aimed to determine the location of floral scent release within the flowers. Given the evidence of hawkmoth pollination in this plant, I aimed to determine whether the chemical composition of *G. scabra*'s floral scent is also consistent with the typical fragrance profile of a hawkmoth-attracting plant, as expected. To the best of our knowledge, this is the first reported analysis of floral volatiles in *G. scabra*, providing a baseline for understanding the chemical ecology in this species.

Methods

Study species

Guettarda scabra (L.) Vent. (Rubiaceae) is an evergreen shrub native to the Caribbean region. It can be found from southern Florida in the United States to northern parts of Colombia and Venezuela (Acevedo-Rodriguez and Strong, 2012; Roberts, 2014; WCSP, 2022). In south Florida, *G. scabra* typically blooms from May to July and is restricted to the few fragments of pine rockland and hardwood hammock habitats remaining today (Tomlinson, 1980). Although this species exhibits a special case of distyly in which stigma and anther height vary continuously, it is still possible to recognize two distinct floral morphs (short- and long-styled flowers) within its populations due to the bimodal distribution of stigma-anther separation (Richards and Koptur, 1993) (Figure 1). The flowers of *G. scabra* are sphingophilous, exhibiting traits usually associated with the attraction of nocturnal moths of the Sphingidae family. These traits include a large, white, tubular corolla with a strong scent, traditionally described as sweet (Faegri and van der Pijl, 1979; Willmer, 2011) (Figure 1). The flowers typically open late in the evening and remain open all night, emitting a strong fragrance that is noticeable from a distance. By the following morning, the scent is detectable only at close range, and the flowers are wilted by noon.

Plant material

During the late afternoon, branches with floral buds ready to open were obtained from long- and short styled-morphs of *G. scabra* growing in two pine rockland fragments in Miami-Dade County, Florida, US: Long Pine Key localized at Everglades National Park (ENP) (25°24’13.2” N 80°39’33.2” W) and at Larry and Penny Thompson Memorial Park (LPT) (25°35’55” N 80°23’55” W). The cut end of each branch was kept in fresh water and immediately transported to the Plant Chemical Ecology Lab at Florida International University. Material from ENP was collected on 22 June 2019 and from LPT on 21 July 2020.

Localization of scent-emitting regions within the flower

To assess the location of osmophores (scent-emitting regions) in both short- and long-styled morphs, detached flowers were stained with neutral red (Vogel, 1990; Dobson et al., 2005), which is selectively absorbed and retained by undamaged osmophore tissue (Effmert et al., 2006). Neutral red is a highly effective stain for osmophores due to two key factors. First, osmophores have a highly permeable cell wall which allows neutral red to enter the cell easily (Effmert et al., 2006). Second, immediately after flower anthesis, osmophores undergo significant changes in their metabolic activity, becoming highly vacuolated to support scent production and release (Stern et al., 1987). Neutral red cations rush into these vacuoles and get locked in them due to the high affinity of vacuoles for positively charged molecules (Effmert et al., 2006). This results in strong staining of osmophores, making them clearly visible.

Ten plants of each floral morph were selected and two freshly-opened flowers were collected from each individual, for a total of 20 flowers per morph. These flowers were submerged in an aqueous solution of neutral red (0.1% in distilled water) for 20 minutes. Afterwards, flowers were rinsed with distilled water to remove excess dye, and then were photographed.

Collection and chemical analysis of floral scents

A preliminary analysis showed that there is no significant difference in the scent collected from uncut flowers in the field and the scent collected from cut flowers in the lab. Therefore, scent samples were collected in the lab to minimize the possibility of contamination and interference in collection efficiency that could arise from daily fluctuations in ambient humidity.

Branches from short- and long- styled floral morphs were collected from 52 individuals: 12 plants/morph at LPT and 14 plants/morph at ENP, to account for variation among individuals. Once in the lab, flowers were carefully removed from branches and grouped according to each floral morph (short- and long-styled) and sampling location (LPT and ENP). Samples from LPT contained 80 flowers per morph (6-10 flowers/plant) and from ENP contained 81 flowers per morph (3-8 flowers/plant). Groups of flowers were placed in 500 ml Erlenmeyer flasks to collect their VOCs using dynamic headspace sampling (Dobson et al. 2005) (Figure 2). Fragrant headspace air in the flask was allowed to reach equilibrium for 15 min, and then air was drawn from the flask using a mini membrane pump (Gilian BDX-II personal air sampling pump, Sensidyne®) at a flow rate of 500 ml/min for 60 min. Air removed from the flask was passed through a 6x70 mm adsorbent glass tube (Zefon International), which contains a mixture of 45 mg Tenax TA® (divided in two sections of 30/15 mg), and 30 mg of active charcoal (mesh 20/40), where VOCs were trapped. Surrounding air was collected simultaneously as negative control, to account for the presence of ambient contaminants (Figure 2). Upon collecting headspace samples, volatiles were eluted from the adsorbent tubes with 1 ml of a solution of hexane and acetone (10:1) and stored at -20 °C until they were analyzed.

Eluted volatile samples were analyzed on a coupled gas chromatography–mass spectrometry system (GC–MS) (7890B/5977A series GC/MSD, Agilent Technologies) equipped with a HP-5ms capillary column (5% phenyl methyl silox; 30 m, 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies). For each sample, 1.2 uL was injected into a 4 mm ID single taper inlet liner with wool (Restek), using a split injection technique (split ratio, 1:1). The carrier gas was helium with a head pressure of 9.7853 psi, a flow rate of 1.2 ml/min flow and the electron impact ion source (EI) was 70 eV, full scan (50–650 amu). Inlet and MSD temperatures were kept constant at 250 °C and 260°C respectively. The GC oven initial temperature was held at 50°C for 2 min, then increased 5°C/min until reaching 75°C, and finally 10°C/min until reaching 240°C, where it was held for 2 min.

VOCs were identified by comparing mass spectral fragmentation patterns with those in the National Institute of Standards and Technology (NIST) libraries using NIST

MS (search program version 2.3). To determine the relative amounts of volatiles in the chromatogram of a sample, the peak area of each compound was calculated as a proportion in relation to the total peak area on that gas chromatogram, allowing comparison among samples. Compounds found in similar abundance in both the control and experimental samples were considered to be contaminants and therefore were excluded from the analysis. Floral compounds found were classified based on Knudsen et al. (2006).

Statistical analysis

Proportions of VOCs present in the floral bouquets of long- and short-styled morph samples were compared using Pearson's chi-square test, or Fisher's exact test (if expected cell frequencies were less than 5) ($p < 0.05$). All analyses were performed using R version 4.1.1 (The R Foundation for Statistical Computing, 2021).

Results

Scent-emitting regions within the flower

The staining experiment showed that the upper surface of the corolla lobes, anthers and stigma in both long- and short-style morphs reacted positively to the neutral red solution, suggesting that flowers of *G. scabra* might emit scent from these parts (Figure 3). The corolla tube, both inside and outside, did not react to the staining and conserved its original color.

Composition of floral scent

A total of 10 dominant VOCs of two chemical classes (benzenoids and terpenes) were found in the scent samples of both long- and short-styled floral morphs of *G. scabra* (Table 1, Figure 4). Floral scent samples of the long- and short-styled morphs had identical VOCs and there was no difference in the relative abundance of each compound (Fisher's exact tests $p > 0.05$ for: linalool, β-caryophyllene, (E,E)-α-farnesene, α-humulene, methyl salicylate, benzyl alcohol. Benzenacetaldehyde (also known as phenylacetaldehyde) $X^2{}_2 = 0.02$, $P = 0.88$; (E)-ß-ocimene $X^2{}_2 = 0.09$, $P = 0.76$; phenylethyl alcohol (also known as 2-phenylethanol) $X^2{}_2 = 0$, $P = 1$; benzaldehyde $X^2{}_2 = 0.35$, $P = 0.55$) (Figures 4 and 5).

The scent of *G. scabra* was strongly dominated by two VOCs, benzenacetaldehyde and (E)-ß-ocimene (Figures 4 and 5), with each of them contributing more than 30% of the total chromatogram area (Figure 5). Phenylethyl alcohol accounted for 8% of the chromatogram area, whereas the remaining compounds were present in smaller relative proportion. Among the 10 VOCs emitted by the flowers, benzyl alcohol was the smallest peak present in the chemical profile, representing less than 0.5% of the total area.

Discussion

Scent emission within the flower

The retention of neutral red in the corolla lobes, anthers, and stigma of both long- and short-styled floral morphs of *G. scabra* suggests that these structures are the primary source of the flower's scent. The presence of scent-emitting regions in flowers is a common trait in the Rubiaceae family, where many species, including *Psychotria homalosperma*

(Watanabe et al., 2018), *Hillia parasitica* (Knudsen and Tollsten, 1993), *Faramea cyanea* (Maruyama et al., 2010), *Isertia laevis* (Wolf et al., 2003), *Kadua haupuensis* (Lorence et al., 2010), and *Randia itatiaiae* (de Avila and Freitas, 2011), produce fragrant flowers whose scent is thought to mediate pollinator attraction. While flower scent production can involve the entire blossom, it is often concentrated in specific regions or structures (Effemert et al., 2006; Willmer, 2010), with petals being generally the main source of VOCs responsible for the fragrance (Dobson et al., 1990; Bergström et al., 1995, Effemert et al., 2006).

The emission of scent by to the corolla lobes in *G. scabra* would be consistent with previous reports of osmophores in this region in other Rubiaceae species, such as *Pagamea duckei* (Terra-Araujo et al., 2012), *Psychotria ipecacuanha* (Rossi et al., 2005), and *Chiococca alba* (Castro et al., 2008). Furthermore, the pattern of staining of the corolla lobe is a trait shared with another member of the genus, *G. platypoda*, albeit in this species neutral red is additionally retained by the corolla tube (Novo et al., 2018). Although non-glandular structures like anthers and stigmata can retain neutral red (Vogel and Hadacek, 2004), their potential contribution to *G. scabra*'s fragrance cannot be dismissed, as they are known to emit odor in many plants (Effemert et al., 2006; Willmer, 2010).

Floral scent is critical for guiding pollinators to flowers at long and short distances (Dobson et al., 2005). However, at close range, scent may also convey information about resource quality for some pollinators (Haverkamp et al., 2016). In the case of *G. scabra*, the upper surface of the corolla lobes and adjacent structures emit fragrance, which is beneficial as it maximizes pollinator exposure to the scent while they feed from the flower. This is particularly important for *G. scabra* since it is pollinated by hawkmoths (Pimienta et al., submitted), whose foraging efforts per flower seem to be heavily influenced by stimulation of the tip of the proboscis with flower volatiles as they reach for nectar inside the corolla (Haverkamp et al., 2016).

Floral scent composition

Although variation in floral scent can occur among groups of plants within a species, such as sexual types, color phenotypes, and floral morphs (Delle-Vedove et al., 2017; Johnson et al., 2019; Liu et al., 2022), my findings show that both long- and short-styled morphs of *G. scabra* have the same composition and proportion of VOCs. This is consistent with observations made in other distylous Rubiaceae, such as *Luculia pinceana* (Wang et al., 2023) and *Psychotria homalosperma* (Watanabe et al., 2018), in which different floral morphs emit the same chemical compounds, albeit some of them with different proportions in *P. homalosperma*. Floral scents with similar chemical profiles across morphs may promote equal rates of visitation by pollinators to either morph, ultimately leading to similar rates of pollination and perhaps promoting outcrossing between them. In this scenario, having similar fragrances could prevent either morph from becoming dominant, resulting in equal abundance of both floral morphs within populations, as observed in *G. scabra* (Richards and Koptur, 1993).

Distantly-related plant species that interact with the same group of pollinators often exhibit evolutionary convergence in their floral traits (pollination syndromes), including the make-up of their flower fragrance (Fenster et al., 2004; Dobson, 2006; Schiestl and Johnson, 2013). The chemical composition of the floral scent of *G. scabra*, as revealed by this study, fits the fragrance profile of plants that commonly attract hawkmoths. This finding is consistent with the recent report that this plant is in fact pollinated by *Xylophanes tersa* (Sphingidae) and likely other nocturnal hawkmoths (Pimienta et al., submitted). Floral scents in many hawkmoth-pollinated plants are generally rich in volatile benzenoids and terpenoids, and often contain small amounts of nitrogen-containing compounds (Knudsen and Tollsten 1993; Miyake et al., 1998; Dobson, 2006). Common constituents of the floral profile of these plants include methyl benzoate, benzyl alcohol, phenylethyl alcohol, and esters such as methyl salicylate among benzenoids, and nerolidol, (E)-ß-ocimene, farnesene, linalool, and β-caryophyllene among terpenoids (Knudsen and Tollsten, 1993; Miyake et al., 1998; Dobson, 2006).

The floral bouquet of *G. scabra* is composed entirely of benzenoids and terpenoids, with two compounds, benzeneacetaldehyde and (E)-ß-ocimene, being the most abundant, followed by phenylethyl alcohol in much lower amounts. These compounds are crucial components in the fragrance of many plant species across different families that depend on hawkmoths for pollination. The codominance of terpenoids and benzenoids found in *G. scabra* is also reported in many other plants, although the primary compounds involved may vary (Dobson, 2006).

Similar to *G. scabra*, (E)-ß-ocimene is a dominant component in the aroma of *Clarkia cocinna* (Onagraceae) (Raguso and Pichersky, 1995), *Brugmansia x candida* (Solanaceae) (Kite and Leon, 1995), *Crinum asiaticum* (Amaryllidaceae) (Miyake et al., 1998), and both *Mirabilis jalapa* and *Selinocarpus parvifolius* (Nyctaginaceae) (Levin et al., 2001; Effmert et al., 2005). In some cases, such as in *Platanthera chlorantha* (Orchidaceae) (Steen et al., 2019), and *Capparis spinosa* (Capparaceae), (E)-ß-ocimene is often codominant with linalool (Kantsa et al., 2023), while in *Dianthus monspessulanus* and *D. superbus* (Caryophyllaceae) this compound shares its dominance with β-caryophyllene (Jürgens et al., 2003). On the other hand, in *Trichosanthes kirilowii* (Cucurbitaceae) the dominant compounds in the fragrance are benzeneacetaldehyde (38.9%) and linalool (23%), a benzenoid and a terpenoid respectively (Miyake et al., 1998).

Some of the floral volatiles found in *G. scabra* are also common in other Rubiaceae that attract sphingid moths as pollinators. For example, the floral essential oil in *Psychotria eurycarpa* is dominated by linalool and methyl salicylate (Setzer et al., 2006a), while in *Randia mutudae* it is largely composed by linalool and benzyl alcohol (Setzer et al., 2006b). On the other hand, phenylethyl alcohol is found in both of these species but in much lower amounts. Interestingly, while the relative abundance of benzyl alcohol among the floral volatiles in *G. scabra* is negligible (<1%), in its close relative *G. poasana* the same compound dominates the composition of the essential oil (77%), while the remaining compounds (cinnamyl alcohol, 1-indanol and phenylethyl alcohol) are less abundant (Lawton et al., 1993). It is very likely that the relative abundance of individual compounds in floral volatiles and essential oils differ because to the nature of the extraction methods used (headspace versus distillation), in fact, some highly VOCs found by headspace method may be absent from the essential oil samples (Dobson, 1991). However, the presence of benzyl alcohol in the floral essential oil of *R. mutudae* and *G. poasana* indicates that VOCs were obtained and are therefore present in the floral fragrance.

Although the floral fragrance of *G. scabra* may enhance its attractiveness to hawkmoth pollinators, it does not preclude visits from other insects capable of using floral volatiles as olfactory cues to locate floral resources. For example, in south Florida, flowers of *G. scabra* are visited at night by two species of long-horned beetles (Cerambycidae) that feed on their pollen (Pimienta and Koptur, 2022). In this case, flower scent is likely used by beetles foraging for pollen, since some anthophilous cerambycids are attracted to linalool (Allison et al., 2004) and to phenylethyl alcohol (Toshova et al., 2016), both compounds present in the fragrance of *G. scabra*. Since *G. scabra* flowers remain open and retain a slight fragrance in the morning, several groups of insects, such as butterflies, wasp, and bees, visit them (Pimienta and Koptur, 2022).

The flowers of *G. scabra* are frequented by diurnal insects who rely predominantly on visual and olfactory cues to find floral rewards. Some of the most assiduous visitors are butterflies *Heliconius charithonia* (Nymphalidae) (Pimienta et al., submitted), who depend strongly on vision to select which flowers to visit from afar, but switch to olfaction once they land on the flower, since their feeding behavior is triggered and heavily modulated by flower scent (Andersson and Dobson, 2003). In fact, the relevance of floral volatiles emitted by *G. scabra* as foraging cues for nymphalid butterflies is also seen in *H. melpomene*, who use benzyl alcohol and linalool to locate food sources (Andersson and Dobson, 2003). Unlike butterflies, bees utilize mostly visual cues when they are close to flowers, but at a distance they are strongly guided by both visual and olfactory cues (Giurfa et al., 1996, Dafni et al., 1997). Many of the species reported visiting flowers of *G. scabra* (Pimienta and Koptur, 2022) are indeed attracted to compounds found in their scent. For example, benzyl alcohol, (E,E)-α-farnesene, and linalool are attractive to *Apis mellifera*, benzeneacetaldehyde to Halictid bees, and methyl salicylate and phenylethyl alcohol attract Euglossini bees (Dötterl and Vereecken, 2010). Most notably, *Euglossa dilemma*, an exotic species found on flowers of *G. scabra* (Pimienta and Koptur, 2022), visits plants in south Florida collecting fragrant compounds that closely resemble those found in its mutualistic orchids in the neotropics (Pemberton and Wheeler, 2006). Some of these compounds include (E)-ß-ocimene, linalool, β-caryophyllene, humulene and (E,E)-α-farnesene, all present in *G. scabra* floral fragrance.

The fragrance profile of *G. scabra* is consistent with the floral-scent bouquets of plants that attract nocturnal moths as pollinators. Likely, some of the compounds present in *G. scabra* flower scent have a dual function: luring pollinators and deterring antagonists. For example, both linalool and β-caryophyllene are known to be attractive to different groups of insect pollinators (Dobson, 2006). At the same time, these compounds have been found to serve also as a deterrent of flower-feeding insects for *Convolvulus arvensis* (Convolvulaceae) and *Melilotus alba* (Fabaceae) (Junker et al., 2010). Similarly, 2-phenylethanol (phenylethyl alcohol) released by *Polemonium viscosum* (Polemoniaceae) at low concentrations serves as an attractant to its pollinator, the bumblebee *Bombus balteatus*; while at high concentrations repels flower-damaging ants (*Formica neorufibarbis*) (Galen et al., 2011). Lastly, in *Petunia x hybrida* methyl benzoate attracts hawkmoths while simultaneously deterring attacks by flower-feeding insects (Kessler et al., 2013).

Conclusions

The fragrance of the flowers of *G. scabra* consists entirely of benzenoid and terpenoid compounds and is characterized by the dominance of benzenacetaldehyde and (E)-ß-ocimene. Its chemical profile fits that of night-blooming plants pollinated by nocturnal hawkmoths. The fact that scent emission comes from the corolla lobes of flowers guarantees its exposure to the hawkmoth’s mouthparts during foraging. This finding suggests that flower scent in *G. scabra* could help boost pollinator's foraging effort, increasing the chance of successful pollen transfer. Despite having two different floral morphs (short- and long-styled), there is no difference in the chemical profiles of their floral scents, indicating that both floral morphs may be attracting the same pollinators equally. While the floral scent in *G. scabra* serves the primary role of attracting nocturnal hawkmoth pollinators, it may be used also by other nocturnal and diurnal insects to locate floral resources. My findings establish a baseline knowledge of the chemical ecology of *G. scabra*. This study represents an example where floral scent chemistry validates predictions based on flower morphology in a sphingophilous plant. A deeper understanding of the traits involved in the association between plants and their pollinators, as presented here, is necessary to develop strategies toward the conservation of plants and pollinators in endangered habitats, such as the pine rockland. Further research in *G. scabra* should address the temporal dynamics of the fragrance release, an unknown and yet relevant aspect of the mutualistic relationship between this plant and their hawkmoth pollinators.

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References

Acevedo-Rodríguez, P., and Strong, M. T. 2012. Catalogue of seed plants of the West Indies. *Smithsonian Contributions to Botany*, 98: 1–1192.

Allison, J., Borden, J. and Seybold, S. 2004. A review of the chemical ecology of the Cerambycidae (Coleoptera). *Evolutionary, Mechanistic and Environmental Approaches to Chemically-Mediated Interactions* 14, 123–150.

Andersson, S. and Dobson, H. E. M. 2003. Behavioral foraging responses by the butterfly *Heliconius melpomene* to *Lantana camara* floral scent. *Journal of Chemical Ecology*, 29: 2303–2318.

Bawa, K. S., Bullock, S. H., Perry, D. R., Coville, R. E. and Grayum, M. H. 1985. Reproductive biology of tropical lowland rain forest trees. II. Pollination systems. *American Journal of Botany*, 72(3): 346–356.

Bergström, G., Dobson, H. E. M and Groth, I. 1995. Spatial fragrance patterns within the flowers of *Ranunculus acris* (Ranunculaceae). *Plant Systematics and Evolution*, 195: 221–242.

Borges, R. M., Somanathan, H. and Kelber, A. 2016. Patterns and processes in nocturnal and crepuscular pollination services. *The Quarterly Review of Biology*, 91(4): 389–418.

Castro, C. C., Oliveira, P. E. A. M. and Pimentel, R. M. 2008. Reproductive biology of the herkogamous vine *Chiococca alba* (L.) Hitchc. (Rubiaceae) in the Atlantic Rain Forest, SE Brazil. *Brazilian Journal of Botany,* 31: 317–321.

Cunningham, J. P., Moore, C. J., Zalucki, M. P. and West, S. A., 2004. Learning, odour preference and flower foraging in moths. *Journal of Experimental Biology*, 207(1): 87–94.

Dafni, A., Lehrer, M. and Kevan, P. G. 1997. Spatial flower parameters and insect spatial vision. *Biological Reviews,* 72(2): 239–282.

de Avila, R. S. and Freitas, L. 2011. Frequency of visits and efficiency of pollination by diurnal and nocturnal lepidopterans for the dioecious tree *Randia itatiaiae* (Rubiaceae). *Australian Journal of Botany*, 59(2): 176–184.

Delle-Vedove, R., Schatz, B. and Dufay, M. 2017. Understanding intraspecific variation of floral scent in light of evolutionary ecology. *Annals of Botany*, 120(1): 1–20.

Dobson, H. E. M. 1991. Analysis of flower and pollen volatiles. *In* Linskens, H. F., and Jackson, J. F. (eds.), Essential oils and waxes: modern methods of plant analysis, 231–251. Springer, Berlin, Heidelberg.

Dobson H. E. M. 1994. Floral volatiles in insect biology. *In* Bernays, E. A. (ed.), Insect–Plant Interactions, vol. 5, 47–81. CRC Press, Boca Raton, FL, USA.

Dobson, H. E. M. 2006. Relationship between floral fragrance composition and type of pollinator. *In* Dudareva, N., and Pichersky, E. (eds.), Biology of floral scent, 147–198. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.

Dobson, H. E. M., Bergström, G. and Groth, I. 1990. Differences in fragrance chemistry between flower parts of *Rosa rugosa* Thunb. (Rosaceae). *Israel Journal of Plant Sciences,* 39 (1-2): 143–156.

Dobson, H. E. M., Raguso, R. A., Knudsen, J. T., and Ayasse, M. 2005. Scent as an attractant. *In* Dafni, A., Kevan, P. G. and Husband, B. C. (eds.), Practical pollination biology, 197– 230. Enviroquest Ltd., Ontario, Canada.

Dötterl, S. and Vereecken, N. J. 2010. The chemical ecology and evolution of bee–flower interactions: a review and perspectives. *Canadian Journal of Zoology*, 88(7): 668–697.

Dudareva, N. and Pichersky, E. 2006. Floral scent metabolic pathways: their regulation and evolution. *In* Dudareva, N. and Pichersky (eds.), Biology of floral scent, 55–78. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.

Dudareva, N., Piechulla, B. and Pichersky, E. 1999. Biogenesis of floral scent. *Horticultural Review*, 24:31–54.

Effmert, U., Buss, D., Rohrbeck, D., and Piechulla, B. 2006. Localization of the synthesis and emission of scent compounds within the flower. *In* Dudareva, N. and Pichersky, E. (eds.) Biology of floral scent, 105–124. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.

Effmert, U., Große, J., Röse, U.S., Ehrig, F., Kägi, R. and Piechulla, B. 2005. Volatile composition, emission pattern, and localization of floral scent emission in *Mirabilis jalapa* (Nyctaginaceae). *American Journal of Botany*, 92(1): 2–12.

Faegri, K., and van der Pilj, L. 1979. The Principles of Pollination Ecology. Pergamon Press, Oxford, UK.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. and Thomson, J. D. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, 35: 375–403.

Florida Natural Areas Inventory (FNAI). 2010. Pine Rockland. *In* Guide to the Natural Communities of Florida, 69–72. Florida Natural Areas Inventory, Tallahassee, FL, USA.

Galen, C., Kaczorowski, R., Todd, S. L., Geib, J. and Raguso, R. A. 2011. Dosage-dependent impacts of a floral volatile compound on pollinators, larcenists, and the potential for floral evolution in the alpine skypilot *Polemonium viscosum*. *The American Naturalist*, 177(2): 258–272.

Giurfa, M., Vorobyev, M., Kevan, P., and Menzel, R. 1996. Detection of coloured stimuli by honeybees: minimum visual angles and receptor specific contrasts. *Journal of Comparative Physiology A*, 178: 699–709.

Haber, W. A. and Frankie, G.W. 1989. A tropical hawkmoth community: Costa Rican dry forest Sphingidae. *Biotropica*, 21: 155–172.

Haverkamp, A., Yon, F., Keesey, I. W., Mißbach, C., Koenig, C., Hansson, B. S., Baldwin, I. T., Knaden, M. and Kessler, D. 2016. Hawkmoths evaluate scenting flowers with the tip of their proboscis. *eLife*, 5: e15039.

Johnson, B. O., Golonka, A. M., Blackwell, A., Azquez, I. V., and Wolfram, N. 2019. Floral scent variation in the heterostylous species *Gelsemium sempervirens*. *Molecules*, 24: 2818.

Jones, I. M., and Koptur, S. 2017. Dead land walking: the value of continued conservation efforts in South Florida's imperiled pine rocklands. *Biodiversity and Conservation*, 26 (14): 3241–3253.

Jones, P. L. and Agrawal, A. A. 2017. Learning in insect pollinators and herbivores. *Annual Review of Entomology*, 62: 53–71.

Junker, R. R., Heidinger, I M. and Blüthgen, N. 2010. Floral scent terpenoids deter the facultative florivore *Metrioptera bicolor* (Ensifera, Tettigoniidae, Decticinae). *Journal of Orthoptera Research*, 19: 69–74.

Jürgens, A., Witt, T., and Gottsberger, G. 2003. Flower scent composition in *Dianthus* and *Saponaria* species (Caryophyllaceae) and its relevance for pollination biology and taxonomy. *Biochemical Systematics and Ecology*, 31: 345–357.

Kantsa, A., Garcia, J. E., Raguso, R. A., Dyer, A. G., Steen, R., Tscheulin, T. and Petanidou, T. 2023. Intrafloral patterns of color and scent in *Capparis spinosa* L. and the ghosts of its selection past. *American Journal of Botany*, 110: e16098.

Kelber, A., 2002. Pattern discrimination in a hawkmoth: innate preferences, learning performance and ecology. *Proceedings of the Royal Society of London*. Series B: Biological Sciences, 269: 2573-2577.

Kessler, D., Diezel, C., Clark, D. G., Colquhoun, T. A. and Baldwin, I. T. 2013. *Petunia* flowers solve the defence/apparency dilemma of pollinator attraction by deploying complex floral blends. *Ecology letters*, 16(3): 299–306.

Kite, G. C. and Leon, C. 1995. Volatile compounds emitted from flowers and leaves of *Brugmansia x candida* (Solanaceae). *Phytochemistry*, 40(4): 1093–1095.

Knudsen, J. T., Eriksson, R., Gershenzon, J. and Ståhl, B. 2006. Diversity and istribution of floral scent. *The Botanical Review* 72: 1–120.

Knudsen, J. T. and Gershenzon, J. 2006. The chemical diversity of floral scent. *In* Dudareva, N. and Pichersky, E. (eds.), Biology of floral scent, 27–52. CRC Press, Taylor & Francis Group, Boca Raton, FL, USA.

Knudsen, J. T. and Tollsten, L. 1993. Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth-pollinated taxa. *Botanical Journal of the Linnean Society*, 113(3): 263–284.

Knudsen, J. T., Tollsten, L. and Bergström, L. G. 1993. Floral scents: a checklist of volatile compounds isolated by head-space techniques. *Phytochemistry*, 33: 253–280.

Koptur, S. 2006. The conservation of specialized and generalized pollination systems in subtropical ecosystems: a case study. *In* Waser N., and Ollerton J. (eds.), Plant–

pollinator interactions: from specialization to generalization, 341–361. University of Chicago Press, Chicago, IL, USA.

Lawton, R. O., Alexander, L. D., Setzer, W. N. and Byler, K. G. 1993. Floral essential oil of *Guettarda poasana* inhibits yeast growth. *Biotropica*, 483–486.

Levin, R. A., Raguso, R. A., and McDade L. A. 2001. Fragrance chemistry and pollinator affinities in Nyctaginaceae. *Phytochemistry,* 58: 429–440.

Liu, C. L., Jia, Y., Li, Y. F., Xiang, Y. F., Pan, Y. Z., Liu, Q. L., Ma, K. H. and Yin, X. C. 2022. The rapid appearance of homostyly in a cultivated distylous population of *Primula forbesii*. *Ecology and Evolution*, 12 (11): e9515.

Lorence, D. H., Wagner, W. L. and Laidlaw, W. G., 2010. *Kadua haupuensis* (Rubiaceae: Spermacoceae), a new endemic species from Kaua'i, Hawaiian Islands. *Brittonia*, 62(2): 137–144.

Maruyama, P. K., Amorim, F. W. and Oliveira, P. E. 2010. Night and day service: Distyly and mixed pollination system in *Faramea cyanea* (Rubiaceae). *Flora-morphology, distribution, functional ecology of plants*, 205(12): 818– 824.

Miyake, T., Yamaoka, R. and Yahara, T. 1998. Floral scents of hawkmoth-pollinated flowers in Japan. *Journal of Plant Research*, 111: 199–205.

Novo, R. R., Consolaro, H., Almeida, N. M. and Castro, C. C., 2018. Floral biology of the velvetseed *Guettarda platypoda* DC. (Rubiaceae): Atypical distyly or style dimorphism?. *Flora-morphology, distribution, functional ecology of plants*, 239: 62–70.

Okamoto, T., Kawakita, A., and Kato, M. 2008. Floral adaptations to nocturnal moth pollination in *Diplomorpha* (Thymelaeaceae). *Plant Species Biology* 23: 192–201.

Pemberton, R.W. and Wheeler, G. S. 2006. Orchid bees don't need orchids: evidence from the naturalization of an orchid bee in Florida. *Ecology*, 87(8): 1995–2001.

Pimienta, M. C. and Koptur, S. 2022. More than moths: Flower visitors of a night-blooming plant in south Florida pine rocklands, USA. *Plants*, 11(20): 2799.

Possley, J., S.W. Woodmansee, and J. Maschinski. 2008. Patterns of plant composition in fragments of globally imperiled pine rockland forest: Effects of soil type, recent fire frequency, and fragment size. *Natural Areas Journal*, 28: 379–394.

Powers, J. M., Seco, R., Faiola, C. L., Sakai, A. K., Weller, S. G., Campbell, D. R. and Guenther, A. 2020. Floral scent composition and fine-scale timing in two moth-pollinated Hawaiian *Schiedea* (Caryophyllaceae). *Frontiers in Plant Science*, 11: 1116.

Raguso, R. A. 2008. Wake up and smell the roses: The ecology and evolution of floral scent. *Annual review of ecology, evolution, and systematics*, 39: 549–569.

Raguso, R. A., and Pichersky, E. 1995. Floral volatiles from *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral scent and moth pollination. *Plant Systematics and Evolution*, 194: 55–67.

Richards, J. H., and Koptur, S. 1993. Floral variation and distyly in *Guettarda scabra* (Rubiaceae). *American Journal of Botany*, 80(1): 31–40.

Riffell, J. A., 2011. The neuroecology of a pollinator's buffet: olfactory preferences and learning in insect pollinators. *Integrative and Comparative Biology*, 51(5): 781–793.

Riffell, J. A., Alarcón, R., Abrell, L., Davidowitz, G., Bronstein, J. L. and Hildebrand, J. G. 2008. Behavioral consequences of innate preferences and olfactory learning in hawkmoth–flower interactions. Proceedings of the National Academy of Sciences, 105(9): 3404–3409.

Roberts, A. 2014. *Guettarda scabra*. The IUCN Red List of Threatened Species 2014: 730 e.T56503696A56503850. https://doi.org/10.2305/IUCN.UK.2014-731 1.RLTS.T56503696A56503850.en. Accessed on 1 April 2023.

Rossi, A. A. B., Oliveira, L. O. D. and Vieira, M. F., 2005. Distyly and variation in floral traits in natural populations of *Psychotria ipecacuanha* (Brot.) Stokes (Rubiaceae). *Brazilian Journal of Botany*, 28: 285–294.

Schiestl, F. P. and Johnson, S. D. 2013. Pollinator-mediated evolution of floral signals. *Trends in ecology and evolution*, 28(5): 307–315.

Setzer, W. N, Noletto J. A and Haber W. A. 2006a. Chemical composition of the floral essential oil of *Psychotria eurycarpa* from Monteverde, Costa Rica. *Journal of essential oil bearing plants*, 9(1): 28–31

Setzer, W. N., Noletto, J. A. and Lawton, R.O. 2006b. Chemical composition of the floral essential oil of *Randia matudae* from Monteverde, Costa Rica. *Flavour and fragrance journal*, 21(2): 244–246.

Steen, R., Norli, H. R., and Thöming, G. 2019. Volatiles composition and timing of emissions in a moth-pollinated orchid in relation to hawkmoth (Lepidoptera: Sphingidae) activity. *Arthropod-Plant Interactions*, 13: 581–592.

Stern, W. L., Curry, K. J. and Pridgeon, A. M., 1987. Osmophores of *Stanhopea* (Orchidaceae). *American Journal of Botany*, 74(9): 1323-1331.

Snyder, J. R., Herndon, A., and Robertson, Jr., W. B. 1990. South Florida rockland. *In* Myers, R. L., and Ewel, J. J. (eds.), Ecosystems of Florida, 230–274. University of Central Florida Press, Orlando, FL, USA.

Terra-Araujo, M. H., Webber, A. C. and Vicentini, A. 2012. Pollination of *Pagamea duckei* Standl.(Rubiaceae): A functionally dioecious species. *Biota Neotropica*, 12, 98–104.

Tomlinson, P. B. 1980. The biology of trees native to tropical Florida. Harvard University Printing Office, Allston, MA, USA.

Toshova, T. B., Subchev, M., Abaev, V., Vuts, J., Imrei, Z., Koczor, S., Galli, Z., van de Ven, R. and Toth, M. 2016. Responses of *Pseudovadonia livida* adults to olfactory and visual cues. *Bulletin of Insectology*, 69(2):161–172.

Vogel, S. 1990. The role of scent glands in pollination, first edition. Smithsonian Institution, Washington, D. C., USA.

Vogel, S. and Hadacek, F., 2004. Contributions to the functional anatomy and biology of *Nelumbo nucifera* (Nelumbonaceae) III. An ecological reappraisal of floral organs. *Plant Systematics and Evolution*, 249 (3), 173–189.

Wang, X., Chen, Y. and Yi, Y., 2023. Features of floral odor and nectar in the distylous *Luculia pinceana* (Rubiaceae) promote compatible pollination by hawkmoths. *Ecology and Evolution*, 13: e9920.

Watanabe, K., Kato, H., Kuraya, E. and Sugawara, T. 2018. Pollination and reproduction of *Psychotria homalosperma*, an endangered distylous tree endemic to the oceanic Bonin (Ogasawara) Islands, Japan. *Plant Species Biology*, 33(1):16–27.

WCSP, 2022. World Checklist of Selected Plant Families. Facilitated by the Royal Botanic Gardens, Kew. Available online: https://wcsp.science.kew.org. Accessed on 19 October 2022.

Willmer, P. 2011. Pollination and floral ecology. Princeton University Press, Princeton, New Jersey, USA.

Wolff, D., Braun, M., and Liede, S. 2003. Nocturnal versus diurnal pollination *success in Isertia laevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds. *Plant Biology*, 5(1): 71–78.

Wright, G. A. and Schiestl, F. P. 2009. The evolution of floral scent: the influence of olfactory learning by insect pollinators on the honest signalling of floral rewards. *Functional Ecology*, 23(5): 841–851.

Tables

Table 1. Relative abundances (%) of floral scent constituents of long- and short-styled flower morphs of *Guettarda scabra* (Rubiaceae) identified in dynamic headspace samples. RT= retention time. Flowers were collected at two sites in south Florida: Long Pine Key, Everglades National Park: (ENP) and Larry and Penny Thompson Memorial Park (LPT). Samples (A) and (B) were obtained from 14 plants and 81 flowers (3-8 flowers/plant) each. Samples (C) and (D) were obtained from 12 plants and 80 flowers (3-6 flowers/plant) each. * Compounds were classified based on Knudsen et al., (2006).

Compound (Class)*	**RT (min)**	**Long-styled morph**		**Short-styled morph**	
		ENP (A)	LPT (C)	ENP (B)	LPT (D)
Benzenoids					
Aldehydes					
benzaldehyde	7.26	5.59	7.76	7.35	2.93
benzeneacetaldehyde	9.25	43.46	32.92	37.64	36.56
Alcohols					
benzyl alcohol	9.09	0.56	0.45	0.18	0.72
phenylethyl alcohol	10.67	11.48	4.79	3.47	12.70
Esters					
methyl salicylate	12.12	0.82	5.81	2.72	8.04
Terpenes					
Monoterpenes					
(*E*)-ß-ocimene	9.34	27.05	37.04	41.04	27.82
linalool	10.39	5.78	1.86	2.75	1.26
Sesquiterpenes					
β-caryophyllene	15.50	1.89	2.78	2.40	2.26
α-humulene	15.94	1.49	2.49	1.71	2.81
(E,E)-α-farnesene	16.52	1.88	4.09	0.74	4.90

Figures

Figure 1. Freshly-opened flowers of *Guettarda scabra*. The stigma in long-styled flowers (left) is clearly visible due to the length of the style supporting it, a characteristic not visible in short-styled morphs (right). Note the naturally occurring reddish color on the outside of the distal portion of the corolla tube.

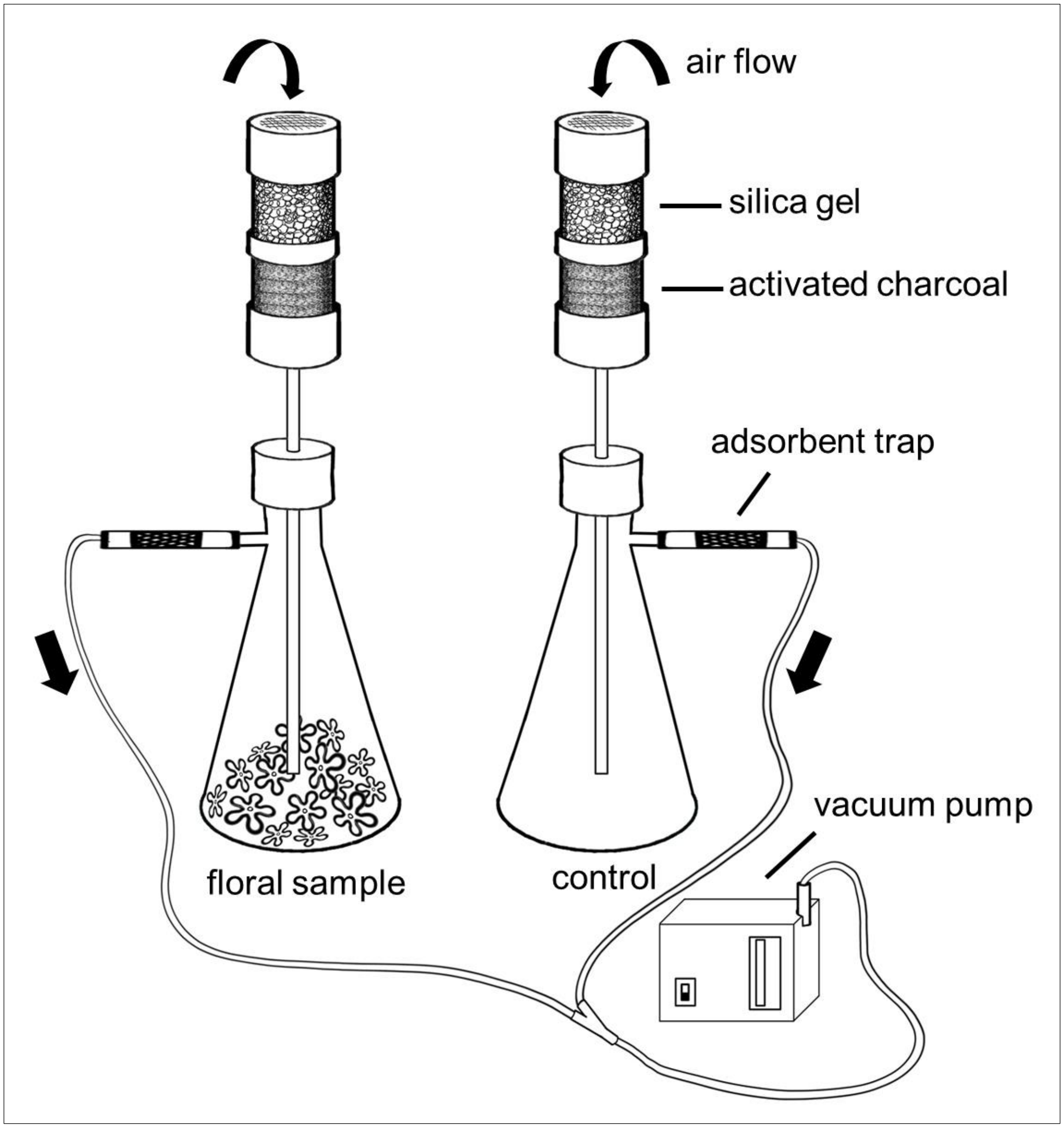

Figure 2. Dynamic headspace setup for the collection of floral scent from detached flowers. Headspace chambers consisted of 500 ml Erlenmeyer flasks into which organic volatile compounds (VOCs) diffused. Using a vacuum pump, air is forced into two flasks, one with the freshly-cut flowers (floral sample) and the other empty (environmental control). Air flowing in is filtered using cartridges containing silica gel and activated charcoal, effectively removing any moisture and contaminants present. The clean air enters the flask and mixes with any VOCs present inside. This enriched air is then forced to flow through adsorbent traps where VOCs are retained, while clean air exits the system through the pump

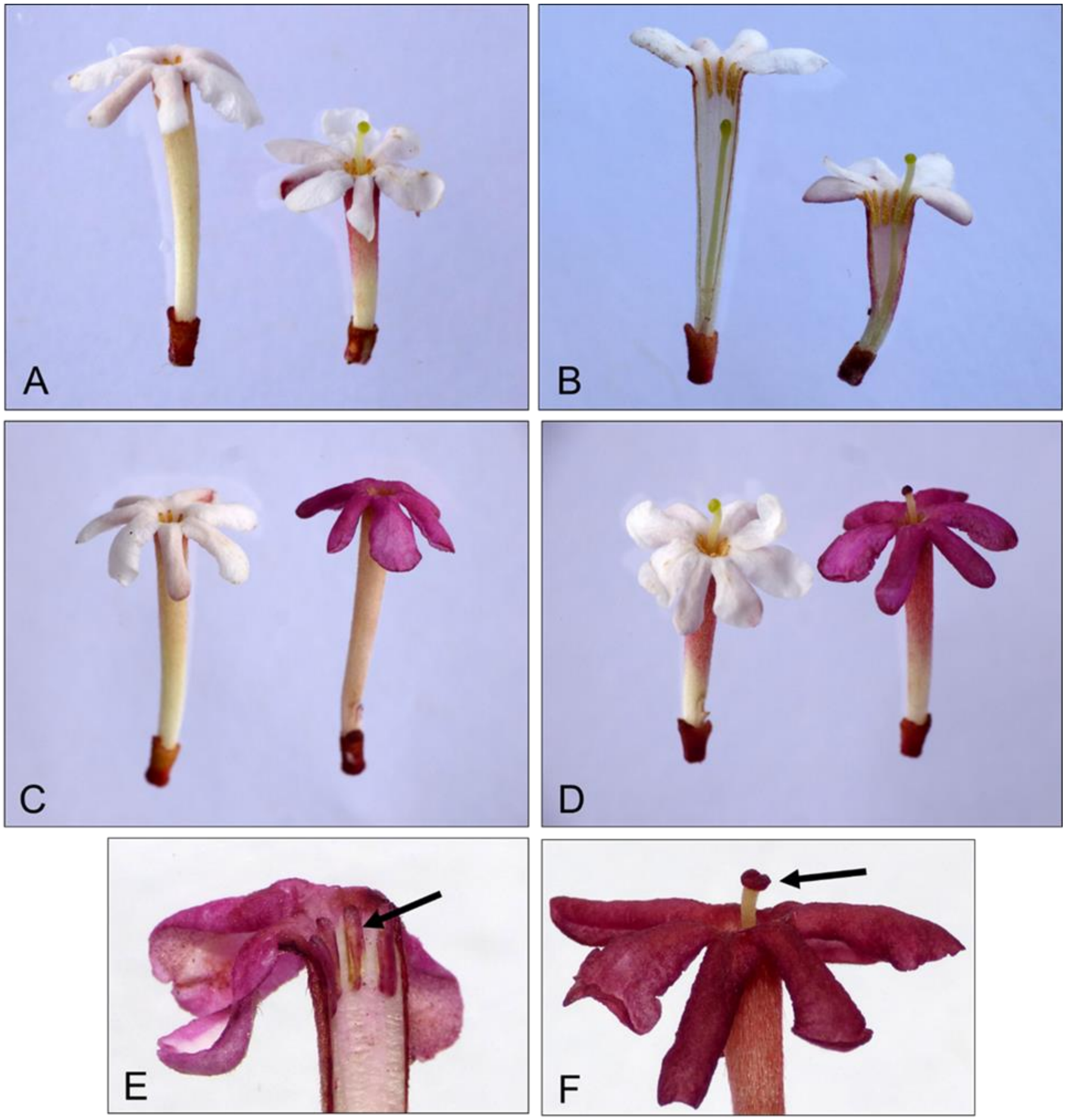

Figure 3. Scent-emitting areas in *Guettarda scabra* floral morphs as evidenced by treatment with neutral red. (A, B) Unstained short- and long-styled morphs. (C) Short-styled flowers before and after staining. (D) Long-styled flowers before and after staining. (E, F) Close up showing the intense purple coloration in petals, anthers, and stigma after having reacted positively with neutral red. The reddish tinge on the outside of the corolla tubes in A and D are not stained but the natural coloring of the pubescence on the outside of some floral tubes.

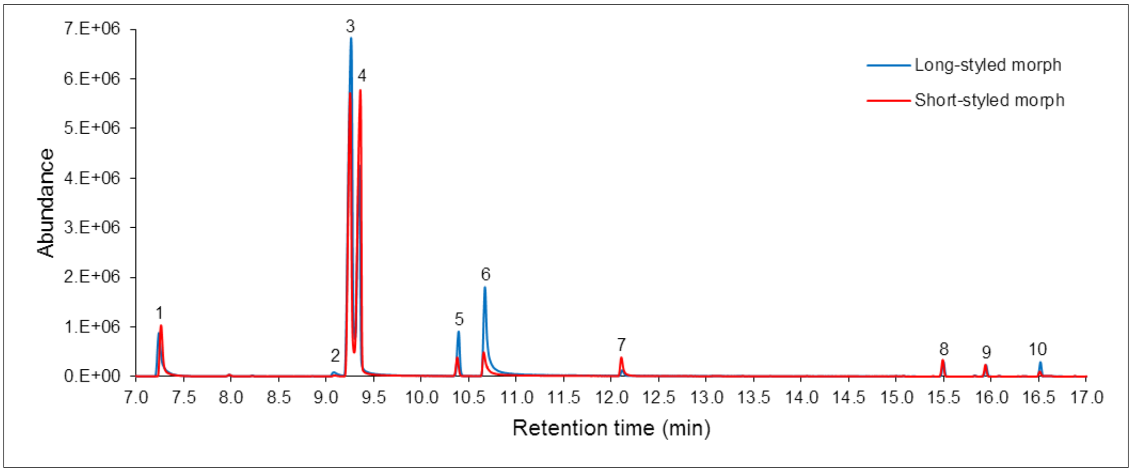

Figure 4. Gas chromatography-mass spectrometry (GC-MS) chromatograms of the volatile organic compounds (VOCs) emitted by long- and short-styled flowers of *Guettarda scabra*. Samples from flowers collected at ENP. Numbered peaks are identified as follows: (1) benzaldehyde, (2) benzyl alcohol, (3) benzenacetaldehyde, (4) (E)-ß-ocimene, (5) linalool, (6) phenylethyl alcohol, (7) methyl salicylate, (8) β-caryophyllene, (9) α-humulene, (10) (E,E)-α-farnesene.

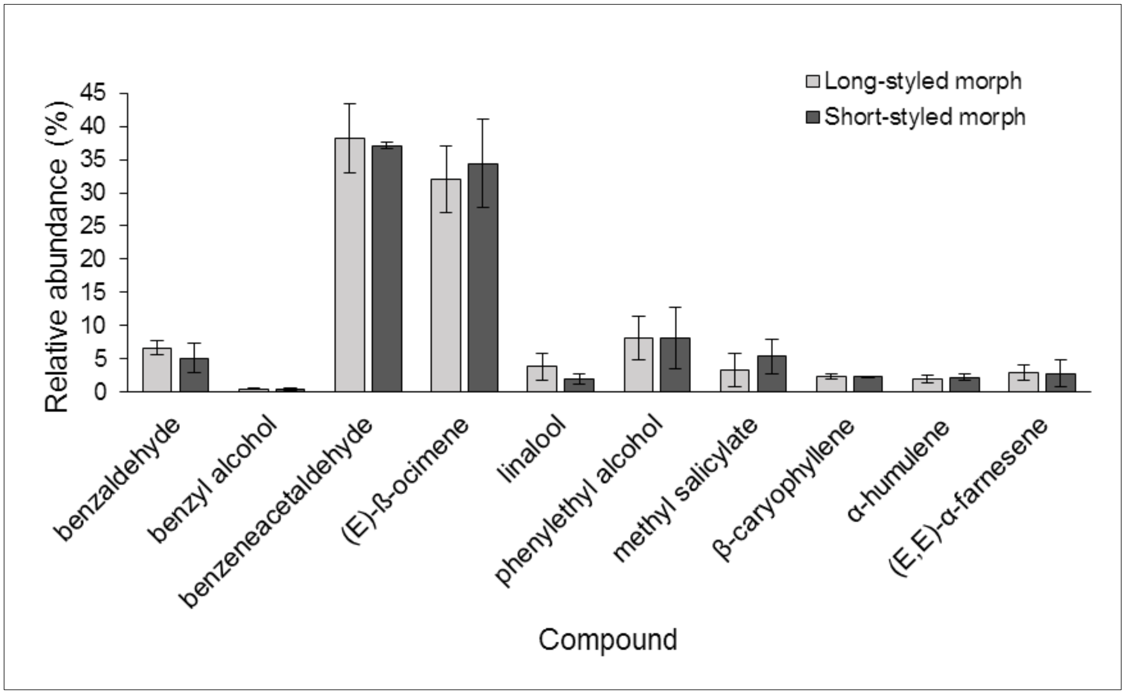

Figure 5. Mean (± SE) of the relative abundance of floral volatile compounds emitted by long- and short-styled morphs of *Guettarda scabra*. $N_{\text{long-styled samples}}$= 2 and $N_{\text{short-styled samples}}$=2.

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTIONS

Knowledge about the floral ecology of native plants is fundamental for effective conservation efforts, particularly when many of these species are at risk from habitat destruction and fragmentation. Both factors can significantly reduce the number of plants and pollinators. A large number of flowering plants depend on insects for successful reproduction, without these pollinators, many species of flowering plants may go extinct.

In south Florida, the pine rockland is a unique habitat containing many plants that depend on insects for their reproduction. Pine rockland has almost disappeared due to development for human use. It is imperative to understand the interactions essential to the survival of plants native to this imperiled ecosystem in order to conserve it.

In my dissertation, I conducted studies to understand some aspects of the floral ecology of the rough-leaf velvetseed *Guettarda scabra* (Rubiaceae), a native species of the south Florida pine rocklands. This species depends on insects for pollen transfer, making it especially vulnerable to habitat loss and fragmentation. By characterizing the insects associated with *G. scabra* flowers I provide baseline data on the local diversity and natural history of insect flower visitors. Although *G. scabra* has floral traits associated with attracting nocturnal moths, my observations show that their flowers are also visited by a much more diverse array of arthropods than that predicted by its pollination syndrome. Most of these visitors rely on floral rewards from *G. scabra* during late summer, when the flowering season has passed for most of the other local plants. Consequently, I find that *G. scabra* plays a critical role in sustaining the local arthropod fauna by providing important foraging and food resources. These natural history observations offer a glimpse of the complexity of plant-animal interactions in general, while providing valuable information on the role of a native species plays in an endangered habitat.

With my exclusion experiment, I tested the importance of the two main groups of visitors on the reproduction of *G. scabra*. I found that *G. scabra* has a specialized pollination system where nocturnal hawkmoths, particularly *Xylophanes tersa*, are the only effective pollinators, while diurnal visitors behave as nectar and pollen thieves. My findings suggest that the dependence on a single and infrequent hawkmoth species to set fruits may put at risk the continuity of local populations of *G. scabra*, especially those confined to suburban pine rockland fragments. This risk increases with the fact that populations of nocturnal hawkmoths, such as *X. tersa,* are likely to be also threatened by fragmentation and the use of pesticides. My findings put in context the critical importance and inherent fragility of specialized pollination systems, where the local collapse of a population of pollinators may disrupt the sexual reproduction of those plants that rely on them for pollen transfer.

My analyses revealed that in this plant, floral fragrance is dominated by benzenacetaldehyde and (E)-ß-ocimene. Interestingly, the chemical profile of the floral scent in *G. scabra* fits that of night-blooming plants pollinated by nocturnal hawkmoths, further supporting the findings of my exclusion experiments. I also found that in this species, flowers emit their fragrance from the corolla lobes, guaranteeing exposure to the hawkmoth's mouthparts during foraging. I proposed that regardless of the floral scent being fine-tuned to the chemosensory system of nocturnal hawkmoths, it may also be used by other nocturnal and diurnal insects to locate floral resources. I also found that, even though *G. scabra* has two different floral morphs, short- and long-styled, there is no difference in the chemical profiles of their floral scents, indicating that both floral morphs may be equally effective in attracting the same pollinators. In general, the floral scent in *G. scabra* serves the primary role of attracting nocturnal hawkmoth pollinators and may help boost their foraging efforts, increasing the chance of successful pollen transfer. My results offer an example where floral scent chemistry validates predictions based on flower morphology.

Further research in *G. scabra* should address whether the high rate of nectar robbery has any effects on the fitness of individual plants. Likewise, it would be also interesting to evaluate the dynamics of the relationship of this plant with other groups of arthropods, besides floral visitors. For example, some of the groups observed visiting the plants during part of this study were predators and could, in theory, have a deterrent effect towards pollinators when located near flowers. However, the same groups could also protect the plant from phytophagous visitors when present among the leaves. Another aspect of the floral ecology of *G. scabra* that remains to be studied is whether the identity of the pollinators and the reproductive success of the plants differs among different fragments of pine rockland habitat. Likewise, it would be important to know if the size of the fragment of habitat has a clear effect on the diversity of the set of pollinators available to the plant. Finally, an unknown and yet relevant aspect of the mutualistic relationship between this plant and their hawkmoth pollinators is the temporal dynamics of the fragrance release. That is, whether the chemical profile of *G. scabra* floral scent varies throughout its anthesis and whether the temporal pattern of emission of its fragrance is in synchrony with the activity of its pollinators. Such behavioral flexibility could have a profound impact on the pollination of a species like *G. scabra*, which flowers open both night and day.

Another potentially fruitful endeavor will be to conduct comparative studies on the smaller-flowered congener, *G. elliptica*, which also occurs in pine rocklands. The greenish flowers also attract insects and their scent profile may differ, perhaps corresponding to a different group of effective pollinators.

VITA

MARIA CLEOPATRA PIMIENTA IDROBO

	Born, Cali, Colombia
2001	B.Sc., Biology Universidad del Valle Cali, Colombia
2018	Tropical Botany Scholarship, Kelly Foundation
2019	Graduate Student Research Grant, FIU Tropics
2019	Graduate Student Research Award, Botanical Society of America
2020	M.Sc., Biology Florida International University Miami, Florida
2020	Best Student Paper Award, Society for Integrative and Comparative Biology
2021	Grants-in-Aid of Research, Society for Integrative and Comparative Biology
2021	Tropical Botany Scholarship, Kelly Foundation
2021	Endowment Grant Research, Florida Native Plant Society
2022	Tropical Botany Scholarship, Kelly Foundation

PUBLICATIONS AND PRESENTATIONS

Valderrama, C., M. C. Pimienta, S. Durán, J. Martínez & P. Olaya. 2004. Biodiversity database of the protected areas in the coffee region. Pp: 71–75, *in*: WCS and WWF Colombia (eds.). Analysis of representation of biodiversity within protected areas in the Coffee region. Cali, Colombia.

Erwin, T. L., M. C. Pimienta, O. Murillo & V. Aschero.2005. Mapping patterns of β-diversity for beetles across the western Amazon Basin: A preliminary case for improving conservation strategies. *Proceedings of the California Academy of Sciences,*56(7): 72–85.

Pimienta, M. C., Montealegre, F., Chacón de Ulloa, P., Gonzalez, R. & Mejía, J. 2008. Diversidad de artrópodos arborícolas en el PNN Gorgona (Cauca, Colombia)/Arboreal arthropod diversity in Gorgona National Park. Pp: 47, *in*: Resúmenes XXXV Congreso de la Sociedad Colombiana de Entomología. Cali, Colombia.

Chacón, P., S. Valdés, A. Hurtado & M. C. Pimienta. 2014. Arboreal ants of Gorgona National Park (Cauca, Colombia). *International Journal of Tropical Biology and Conservation*, vol. 62 (Suppl.1): 277–287

Montealegre, F., F. A. Sarria, M. C. Pimienta & A. C. Mason. 2014. Arboreal katydids from Gorgona National Park, Colombia (Orthoptera: Tettigoniidae). *International Journal of Tropical Biology and Conservation*, vol. 62 (Suppl.1): 289–296.

Erwin, T. L. & M. C. Pimienta. 2018. Biological Monitoring Insects, Volume 6, Arboreal Insects. Ecuambiente Consulting Group, Quito, Ecuador.

Pimienta, M. C. & Koptur S. 2018. A preliminary survey of *Guettarda scabra* (Rubiaceae) flower-visiting insects in south Florida. Poster presented at the Pine Rockland and Tropical Botany Conference, Fairchild Tropical Botanic Garden, Miami, FL.

Ruiz, C., & Pimienta, M. C. 2019. Behavior of adult *Leucopis* sp. (Chamaemyiidae) associated with aphids feeding on flowers of the rough-leaf velvetseed (*Guettarda scabra*: Rubiaceae) in south Florida. *Fly Times*, 62: 8–10.

Pimienta, M. C. & Koptur S. 2019. Floral visitors of the rough-leaf velvetseed *Guettarda scabra* (Rubiaceae) in south Florida. Poster presented at the Botanical Society of America, Botany, Tucson, AZ.

Pimienta, M. C., Ruiz, C. & Koptur S. 2020. Do diurnal floral visitors increase the fruit-set of a sphingophilous plant?: The case of the rough-leaf velvetseed *Guettarda scabra* (Rubiaceae). Poster presented at the Society for Integrative & Comparative Biology, meeting held in Austin, TX.

Koptur, S., J. Clayborn, B. Harris, I. Jones, M. C. Pimienta, A. S. Primoli, & P. S. Oliveira. 2022. Caterpillar responses to ant protectors of plants. P.p: 297–317 in: R. J. Marquis & S. Koptur (eds.). Caterpillars in the middle: Tritrophic interactions in a changing world. Springer, Cham.

Pimienta, M. C. and Koptur, S. 2022. More than moths: Flower visitors of a night-blooming plant in south Florida pine rocklands, USA. *Plants*, 11: 2799.

Koptur, S., Primoli, A. S. and Pimienta, M. C. 2023. Defoliation in perennial plants: Predictable and surprising results in *Senna* spp. *Plants*, 12:587.