UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Botucatu

EFEITOS DA FLORIVORIA SOBRE A SINALIZAÇÃO QUÍMICA E VISUAL EM INTERAÇÕES PLANTA-POLINIZADOR

PRISCILA TEIXEIRA TUNES

Tese apresentada ao Instituto de Biociências, Câmpus de Botucatu, UNESP, para obtenção do título de Doutorado no Programa de Pós-Graduação em Ciências Biológicas (Botânica), Área de concentração Morfologia e Diversidade Vegetal.

Botucatu – SP

2022

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ORIENTADORA: PROFª DRª ELZA GUIMARÃES

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1. Compostos orgânicos voláteis. 2. Comunicação visual. 3. Herbivoria. 4. Polinização.

Palavras-chave: Area floral; Compostos orgânicos voláteis (COVs) florais; Comunicação química; Comunicação visual; Florivoria.

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SUMÁRIO

TUNES, P. **EFEITOS DA FLORIVORIA SOBRE A SINALIZAÇÃO QUÍMICA E VISUAL EM INTERAÇÕES PLANTA-POLINIZADOR.** 2022. 168p. TESE (DOUTORADO) – INSTITUTO DE BIOCIÊNCIAS, UNESP – UNIVERSIDADE ESTADUAL PAULISTA, BOTUCATU.

RESUMO - Interações bióticas demandam o estabelecimento prévio de vias de comunicação planta-animal, as quais são essencialmente moduladas por sinais visuais e químicos. Dentre os caracteres relacionados à polinização, a cor, o padrão de coloração, a forma floral e a emissão de compostos voláteis florais desempenham papel fundamental na atração dos polinizadores. Entretanto, as pistas visuais e químicas podem ser utilizadas para a localização das flores não apenas pelos polinizadores, mas também por florívoros, sendo que a atuação de ambos pode ser simultânea e conflitante. A interface florívoro-flor-polinizador, ainda pouco explorada, constitui-se em um sistema apropriado e bastante interessante para o entendimento das complexas interações envolvendo angiospermas e seus visitantes florais. Esse cenário indica a importância de ampliarmos e aprofundarmos estudos envolvendo interações múltiplas para alcançarmos uma compreensão mais consistente sobre o processo de polinização animal, que media a reprodução de cerca de 94% das espécies vegetais tropicais. Há lacunas substanciais nas pesquisas sobre a ocorrência da florivoria em sistemas naturais, especialmente sobre os fatores ambientais e ecológicos que modulam esta interação e seus efeitos sobre a polinização e reprodução das plantas. Apesar de haver um crescente número de publicações que têm se concentrado nos efeitos da florivoria sobre o sucesso reprodutivo vegetal, são poucos os estudos que exploram as modificações decorrentes da florivoria nos atrativos florais químicos e visuais e nas vias de comunicação entre planta e polinizador. Nesse sentido, o presente estudo visa ampliar o conhecimento sobre as bases das interações florívoro-planta-polinizador. Assim, apresentamos cinco capítulos em que exploramos, de forma complementar, diversos aspectos da interação florívoro-flor-polinizador, envolvendo a incidência da florivoria, seus possíveis moduladores ambientais, seu impacto sobre os atrativos florais químicos e visuais, e seu o impacto sobre a polinização de espécies vegetais tropicais. Em síntese, verificamos que uma ampla gama de espécies vegetais sofre florivoria e que a ação dos florívoros afetou os caracteres químicos e visuais de maneira espécie-específica, sendo que, na maioria dos casos, a florivoria não afetou as características do odor floral. Adicionalmente, constatamos que a simetria floral, a área

floral e a integridade de seu contorno não são essenciais para a manutenção da interação com abelhas polinizadoras. Deste modo, concluímos que os efeitos da florivoria sobre atrativos florais isolados pode não ser suficiente para gerar uma resposta comportamental dos polinizadores que seja prejudicial à polinização, especialmente porque diversos grupos de polinizadores se utilizam de pistas multimodais para forragear e visitar flores. Nosso estudo destaca a complexidade intrínseca às interações florívoro-flor-polinizador e ressalta a necessidade de aprofundarmos nossos conhecimentos sobre o tema para que possamos desvendar e compreender padrões macroecológicos de florivoria e suas possíveis consequências para a comunicação flor-polinizador e, consequentemente, para a polinização das espécies vegetais.

Palavras-chave: área floral, compostos orgânicos voláteis (COVs) florais, comunicação química, comunicação visual, florivoria, simetria floral, herbivoria floral, polinização.

ABSTRACT

Biotic interactions demand the previous establishment of plant-animal communication pathways, which are essentially modulated by visual and chemical cues. Among the traits related to pollination, floral colour, colour patterns, floral shape and the emission of floral volatile compounds present a crucial role on pollinator attraction. However, the visual and chemical cues can be used to locate flowers not only by the pollinators, but also by florivores, being that both can act concomitantly and contrastingly. The florivore-flower-pollinator interface, still poorly explored, constitutes an appropriate and interesting system to understand complex antagonist-plant-mutualist interactions. This scenario highlights the importance of broadening and deepening studies regarding multiple interactions to reach a more consistent comprehension of the process of animal pollination, which mediates the reproduction of approximately 94% of tropical plant species. There are substantial gaps in the research on the occurrence of florivory in natural systems, especially regarding the environmental and ecological factors that drive this interaction and its effects on plant species pollination and reproduction. Besides the growing number of papers focussing on the effects of florivory on plant reproductive success, there are few studies that explore the changes induced by florivory on floral chemical and visual attractants and on plant-pollinator communication pathways. Therefore, the present study aims to help to fill several existing gaps in the study of florivore-flower-pollinator interactions. Thus, we present five chapters in which we explore, in a complementary manner, several facets of the florivore-flower-pollinator interaction, involving the incidence of florivory, its possible environmental drivers, its effect on floral visual and chemical attractants, and its effect on tropical plant species pollination. In summary, we verified that an ample array of plant species shows signs of florivory, however, florivore's effect on floral chemical and visual attractants was species-specific, being that, in most cases, florivory did not affect chemical attractants. Additionally, we verified that floral symmetry, floral area and its outline integrity are not essential for the maintenance of plant-pollinator interactions. Therefore, we conclude that the effects of florivory on single floral attractants might not be enough to trigger a pollinator behavioural response that is detrimental to pollination, especially because many pollinator groups rely on multimodal cues to make their foraging decisions and visit flowers. Our study showcases the intrinsic complexity of florivore-flower-pollinator interactions and highlights the need to broaden our knowledge regarding this subject to unravel and understand macroecological patterns of florivory and its possible

consequences for flower-pollinator communication and, consequently, for plant species reproduction.

Keywords: chemical communication, floral area, floral herbivory, floral symmetry, floral volatile organic compounds (VOCs), florivory, pollination, visual communication.

INTRODUÇÃO GERAL

Os processos de evolução e radiação das Angiospermas e dos diversos grupos de insetos ocorrem de modo entrelaçado (Labandeira, 2001; Labandeira, 2002). Os animais usam uma diversidade de caracteres florais como pistas para localizar flores no ambiente (Schaefer e Ruxton, 2011; Willmer, 2011) e tais características podem ser moduladas por interações com florívoros (consumidores de flores) e polinizadores (Andrews *et al*., 2007, Jogesh *et al*., 2017). Florívoros podem afetar o sucesso reprodutivo das plantas diretamente ao se alimentarem de estruturas reprodutivas (por exemplo, óvulos e pólen); ou indiretamente, alimentando-se de verticilos florais estéreis, alterando caracteres florais e potencialmente afetando a atração e a visitação dos polinizadores (McCall e Irwin, 2006). Dentre os caracteres florais relacionados à polinização, a cor e os padrões de coloração (Lunau *et al*., 2011; Schaeffer e Ruxton 2011; Papiorek *et al*., 2016), a forma floral (Willmer, 2011) e a emissão de compostos voláteis florais (Dudareva *et al*. 2006) desempenham papel fundamental, isolada ou conjuntamente, na atração dos polinizadores. Adicionalmente, é importante ressaltar que, além de estes sinais serem emitidos adequadamente pelas flores, eles precisam ser discriminados pelo polinizador a partir de suas capacidades sensoriais (Chittka e Thomson, 2001; Knauer e Schiestl, 2015) para que a interação se estabeleça.

Os efeitos diretos da florivoria sobre a reprodução das plantas são facilmente mensuráveis, já que são decorrentes da depleção das estruturas reprodutivas em si; entretanto, os efeitos indiretos são mais difíceis de determinar, pois envolvem potenciais modificações na comunicação planta-polinizador, as quais podem levar os polinizadores a negligenciarem as flores danificadas (Mothershead e Marquis, 2000 e referências; Malo *et al*., 2001). Estudos sobre as implicações evolutivas das interações ecológicas geralmente avaliam os efeitos par a par (Irwin, 2006), como por exemplo, o modelo da

diversificação mediada por polinizadores (van der Niet e Johnson, 2012). Entretanto, estudos recentes têm investigado as pressões seletivas exercidas por distintos grupos de animais antófilos, como os florívoros, além dos polinizadores (Caballero *et al*., 2013, Jogesh *et al*., 2017). Há lacunas substanciais nas pesquisas sobre a ocorrência da florivoria em sistemas naturais, especialmente sobre os fatores ambientais e ecológicos que modulam esta interação e seus efeitos sobre a polinização e reprodução das plantas (Haas e Lortie, 2020). Em uma abordagem complementar Boaventura *et al*. (2021) propõem uma sistematização dos padrões globais enfocando como os caracteres florais podem afetar os danos florais visando compreender a dinâmica eco-evolutiva das interações florívoro-flor.

Há um crescente número de publicações que tem se concentrado nos efeitos da florivoria sobre o sucesso reprodutivo vegetal (Tabela 1). Esses estudos revelam resultados variados, incluindo negativos (McCall, 2008; Kessler e Halitschke, 2009; Tsuji *et al*., 2016), neutros (Mothershead e Marquis, 2000; Leavitt e Robertson, 2006; Sober et al., 2009; Tunes *et al*., *in prep*, Capítulo 4 desta tese) e até mesmo efeitos positivos da florivoria sobre sucesso reprodutivo da planta em alguns cenários específicos (Botto-Mahan *et al*., 2011). Esse panorama indica a importância de ampliarmos e aprofundarmos estudos envolvendo interações múltiplas para alcançarmos uma compreensão mais consistente sobre o processo de polinização animal, que media a reprodução de cerca de 94% das espécies vegetais tropicais (Ollerton et al., 2011).

Os 90 estudos envolvendo florivoria levantados na Tabela 1, englobam mais de 70 espécies vegetais distintas e os florívoros associados a elas. Entretanto, é notável que os estudos em sua maioria englobam uma espécie ou um pequeno conjunto de espécies vegetais, faltando estudos que explorem a florivoria em um contexto de comunidade. Adicionalmente, aproximadamente 45% dos trabalhos focam no efeito da florivoria sobre

Tabela 1. Relação dos estudos envolvendo florivoria publicados em periódicos científicos incluindo a(s) espécie(s) vegetal(is) amostrada(s), seus florívoros, a variável referente à florivoria mensurada em cada estudo e os principais enfoques dos mesmos. Os artigos compilados nessa tabela estão listados no 'Google Scholar' no período de 1945 a 2021 contendo termos-chave (florivor* OU florivore* OU "flower antagonis*" OU "floral damage" OU "flower damage" OU herbivor* E pollinat* OU herbivor* E flower*). BR= biologia reprodutiva, DF= medidas de danos florais, DO= defesa ótima, NA = informação não apresentada no estudo original, O= outro, RN= roubo de néctar, SR= sucesso reprodutivo.

Espécie vegetal	Florívoros	Florivoria	Foco do estudo	Referência
Castilleja indivisa	Lagartas de mariposas	Frequência de ataques	SR	Adler *et al*., 2001
Castilleja indivisa	Lagartas de mariposas	Frequência de ataques	SR	Adler, 2000
Brassica napus	Besouros e lagartas	NA	SR	Åhman *et al*., 2009
Banisteriopsis malifolia	Besouros	Frequência de ataques	O	Alves-silva *et al*., 2015
Banisteriopsis malifolia	Lagartas	Frequência de ataques	O	Alves-silva *et al*., 2018
Fragaria virginiana	Besouros	Frequência de ataques	DF BR	Ashman *et al*., 2004
Eutrochium dubium	Lagartas e larvas de moscas e besouros	Frequência de ataques	DF	Bertin *et al*., 2010
Diversas espécies	Diversos grupos incluindo vertebrados e invertebrados	Frequência de ataques e % removida da área das flores	O	Boaventura *et al*., 2021
Protea neriifolia	Roedores	Frequência de ataques	DF	Botha e Pauw, 2017
Alstroemeria ligtu va. Simsii	Lagartas de Lepidoptera e larvas de besouros	Frequência de ataques	SR	Botto-Mahan *et al*., 2011
Impatiens capensis	Besouros e gafanhotos	% removida da área das flores	DF	Boyer *et al*., 2016
Viola riviniana	Lesmas, besouros e lagartas	% removida da área das flores	DF	Breadmore e Kirk, 1998
Tristerix aphyllus	NA	Frequência de ataques	SR	Caballero *et al*., 2013
Aphelandra aurantiaca	Besouros, lagartas e gafanhotos	Frequência de ataques	SR	Calvo-Irabién e Islas-Luna, 1999
Aechmea pectinata	Caranguejo	Frequência de ataques	DF SR	Canela e Sazima, 2003

Centrosema virginianum	Besouros	Frequência de ataques	SR	Cardel e Koptur, 2010
Loasa tricolor	Lagartas de Lepidoptera e larvas de besouros	% removida da área das flores	SR	Cares-Suarez *et al*., 2011
Dryas octopetala	Renas	Frequência de ataques	SR	Cooper e Wookey, 2003
Calyptrogyne ghiesbreghtiana	Esperanças	Frequência de ataques	BR SR	Cunningham, 1995
Eryngium yuccifolium	Lagartas de mariposas	NA	SR	Danderson e Molano-Flores, 2010
Ursinia calenduliflora	Besouros	Frequência de ataques	DF SR	de Jager e Ellis, 2014
Turnera sp.	NA	Frequência de ataques	BR	Dutton *et al*., 2016
Arctostaphylos pungens	Lagartas	Frequência de ataques	SR DF RN	Eliyahu *et al*., 2015
Caladenia rigida	Aves	Frequência de ataques	DF	Faast e Facelli, 2009
Banisteriopsis malifolia	Lagartas de Lepidoptera e tesourinhas	% removida da área das flores	SR	Ferreira e Torezan-Silingardi, 2013
Acacia nigrescens	Girafas	% removida da área das flores	DF	Fleming *et al*., 2006
Anemone multifida	Lagartas	Frequência de ataques	SR DF	Gavini *et al*., 2019
Iris hexagona	Cervídeos	Frequência de ataques	DF	Geddes e Mopper, 2006
Silene ciliata	Besouros	Frequência de ataques	BR	Gimenez-Benavides *et al*., 2008
Echinopsis rhodotricha	Cervídeos e porcos	Frequência de ataques	DF	Gomes *et al*., 2016
Impatiens capensis	Gafanhotos, esperanças e besouros	Frequência de ataques	DF RN	Gorden e Adler, 2013
Impatiens capensis	Gafanhotos, esperanças e besouros	% removida da área das flores	DF RN	Gorden e Adler, 2016
Diversas espécies	Lagartas de Lepidoptera, besouros, larvas de besouros e larvas de moscas	NA	SR O	Haas e Lortie, 2020
Taraxacum officinale	Lesma	Frequência de ataques	DF	Honěk e Martinková, 2014
Gelsemium sempervirens	Lagartas	Frequência de ataques	SR DF RN	Irwin *et al*., 2014
Gelsemium sempervirens	Lagartas	Frequência de ataques	SR DF RN	Irwin *et al*., 2018
Oenothera toumeyi	Lagartas	Frequência de ataques	NA	Jogesh *et al*., 2017
Peraxilla tretrapetala	Lagartas	Frequência de ataques	DF	Kelly *et al*., 2008
Nicotiana attenuata	Lagartas	Frequência de ataques	BR DF RN	Kessler *et al*., 2008

Polemonium viscosum	Besouros e gafanhotos	Frequência de ataques	BR DF RN	Kessler *et al*., 2013
Isomeris arborea	Larvas de besouros	NA	SR	Krupnick e Weis, 1999
Isomeris arborea	Larvas de besouros	Frequência de ataques	SR	Krupnick *et al*., 1999
Rafflesia sp.	Carnívoros, cervídeos, macacos, pangolins e roedores	Frequência de ataques	DF	Kusuma *et al*., 2018
Lepidium papilliferum	Besouros e lagartas	NA	SR	Leavitt e Robertson, 2006
Pedicularis gruina	Lagartas	Frequência de ataques	SR	Liao *et al*., 2013
Pastinaca sativa	Lagartas de mariposas	NA	SR	Lohman *et al*., 1996
Satyrium ciliatum	Larvas	Frequência de ataques	BR	Lu *et al*., 2012
Brassica nigra	Lagartas de Lepidoptera	NA	SR	Lucas-Barbosa *et al*., 2013
Yucca baccata	Gado	Frequência de ataques	SR	Lybbert e St Clair, 2017
Philodendron bipinnatifidum	Besouros	Frequência de ataques	DF	Maldonado *et al*., 2015
Myrmecophikz tibicinis	Formigas	Frequência de ataques	DF	Malo *et al*., 2001
Opuntia macrocentra	Lagartas	Frequência de ataques	BR	Mandujano, 2013
Anemone nemorosa	Cervídeo e roedores	% removida da área das flores	DF	Mårell *et al*., 2009
Ariocarpus fissuratus	Besouros	Frequência de ataques	BR	Martínez-Peralta e Mandujano, 2011
Nemophila menziesii	Lagartas de Lepidoptera	Frequência de ataques	DF	McCall e Barr, 2012
Nicotiana attenuata	Lagartas	Frequência de ataques	DO	McCall e Karban, 2006
Raphanus sativus	Lagartas, tesourinhas e besouros	Frequência de ataques	DF	McCall *et al*., 2013
Nemophila menziesii	Lagartas	Frequência de ataques	SR O	McCall, 2008
Nemophila menziesii	Lagartas	Frequência de ataques	SR	McCall, 2010
Mimulus guttatus	Gafanhotos e besouros	Frequência de ataques	DF	Meindl *et al*., 2013
Heliconia spathocircinata	Larvas de moscas	Frequência de ataques	SR	Missagia e Alves, 2017
Oenothera macrocarpa	Gafanhotos e besouros	Frequência de ataques	SR	Mothershead e Marquis, 2000
Silene germana	Lagartas	Frequência de ataques	BR SR	Narbona *et al*., 2018
Macleania bullata	Besouros	Frequência de ataques	SR RN	Navarro, 2001
Iris gracilipes	Lagartas	Frequência de ataques	SR	Oguro e Sakai, 2009
Adenocaulon himalaicum	Lagartas, larvas e lesmas	Frequência de ataques	SR	Oguro e Sakai, 2015

Iris gracilipes	Lagartas	Frequência de ataques	DO	Onodera *et al*., 2014
Tillandsia achyrostachys	Lagartas	Frequência de ataques	DF SR	Orozco-Ibarrola *et al*., 2015
Tillandsia carlos-hankii	Roedores	Frequência de ataques	DF	Palacios-Mosquera *et al*., 2019
Tragopogon dubius	Roedores	Frequência de ataques	O	Pearson *et al*., 2012
Gesneria pauciflora	Lagartas	Frequência de ataques	BR SR	Pérez *et al*., 2018
Opuntia microdasys	Lagartas	Frequência de ataques	BR DF	Piña *et al*., 2010
Bletia patula	Besouros	% removida da área das flores	O	Recart *et al*., 2013
Glycine max	Lagartas	% removida da área das flores	DF	Reisig *et al*., 2017
Phlox hirsuta	Besouros	Frequência de ataques	SR	Ruane *et al*., 2014
Digitalis purpurea	Lagartas	Frequência de ataques	DF	Sletvold e Grindeland, 2008
Verbascum nigrum	Besouros	Frequência de ataques	SR	Sõber *et al*., 2010
Verbascum nigrum	Besouros	Frequência de ataques	SR	Sõber *et al*., 2009
Ipomoea hederifolia	Lagartas	Frequência de ataques	DF SR	Sowell e Wolfe, 2010
Pinguicula moranensis	Formigas e vespas	Frequência de ataques	DF	Suárez-Piña *et al*., 2016
Brassica napus	Besouros	NA	SR	Sutter e Albrecht, 2016
Ageratum conyzoides	Gafanhotos	Frequência de ataques	DO	Tan e Tan, 2017
Cistus ladanifer	Formigas e besouros	Frequência de ataques	DF	Teixido *et al*., 2011
Iris hexagona	Cervídeo	Frequência de ataques	DF	Tobler *et al*., 2006
Eurya japonica	Lagartas	Frequência de ataques	DF	Tsuji e Sota, 2010
Eurya japonica	Lagartas	Frequência de ataques	DF	Tsuji e Sota, 2013
Mimulus aurantiacus	Esperanças, gafanhotos e lagartas	Frequência de ataques	DF	Tsuji *et al*., 2016
Solanum carolinense	Roedores e lagarta de Lepidoptera	Frequência de ataques	BR	Wise e Hébert, 2010
Iris bulleyana	Vespas	Frequência de ataques	SR DF RN	Ye *et al*., 2017
Pastinaca sativa	Lagartas de mariposas	NA	SR	Zangerl e Berenbaum, 2009

o sucesso reprodutivo das espécies vegetais e/ ou focam nas medidas dos danos florais em si. Apenas 11% dos estudos utilizam medidas referentes à porcentagem das áreas das flores removida pelos florívoros. Tal medida aporta informações extremamente relevantes sobre como os florívoros podem afetar a reprodução das espécies vegetais, uma vez que a remoção completa ou parcial das flores por estes animais tem significados biológicos distintos (McCall e Irwin, 2006 e referências). No primeiro cenário, ao consumir completamente as flores, o florívoro necessariamente afeta a reprodução da espécie vegetal, pois erradica a chance de que a flor consumida se torne um fruto. Por outro lado, ao consumir parcialmente as flores, o florívoro não erradica diretamente a chance de tal flor se tornar um fruto, pois a flor ainda pode ser visitada e polinizada, mas, nesse cenário, é possível que a florivoria deflagre algum tipo de resposta por parte do polinizador. É notável que, apesar do crescente número de publicações sobre os efeitos da florivoria sobre a reprodução das espécies vegetais, a grande maioria dos estudos foca exclusivamente no resultado dessa interação, ou seja, em componentes do sucesso reprodutivo em si. São poucos os estudos que exploram as modificações decorrentes da florivoria sobre os caracteres químicos e visuais e sobre as vias de comunicação entre planta e polinizador, buscando identificar os mecanismos subjacentes ao resultado final da interação.

Nesse sentido, o presente estudo visa ampliar nosso entendimento sobre as interações florívoro-planta-polinizador. No Capítulo 1, caracterizamos a florivoria em uma comunidade de cerrado e exploramos fatores ecológicos que poderiam atuar como possíveis moduladores da florivoria em uma comunidade de cerrado. Investigamos se a florivoria é influenciada pela distribuição espacial das espécies de plantas, pela distribuição espacial das unidades de atração do polinizador, pelo tamanho da unidade de atração do polinizador, pelo tipo de unidade de atração do polinizador e pela relação

filogenética entre as espécies vegetais da comunidade. Já no Capítulo 2, investigamos se tais mudanças de simetria e área da corola causadas pela florivoria afetam o comportamento de abelhas polinizadoras. Por sua vez, no Capítulo 3, identificamos as áreas da corola que correspondem aos osmóforos (estrutura secretora responsável pela emissão do odor floral), detalhamos sua estrutura em espécies de plantas com diferentes sistemas de polinização. Assim, comparamos a porção floral portadora de osmóforos com o padrão natural da florivoria, com o objetivo de identificar se os florívoros estão consumindo essas porções, e investigamos se a florivoria afetava a quantidade total de odor floral emitido pelas flores consumidas devido à remoção do tecido secretor. No Capítulo 4, apresentamos um estudo focado na resposta química local das flores à florivoria natural e em como esta poderia afetar os atrativos químicos florais em espécies vegetais com distintos sistemas de polinização. Por fim, no Capítulo 5, pretendemos elucidar se a florivoria afeta concomitantemente os atrativos visuais e químicos, o recurso de néctar e, finalmente, o sucesso da polinização em uma espécie vegetal polinizada por beija-flores. Neste capítulo, abrangemos todos os componentes da interação florívoro-flor-polinizador, desde os níveis de florivoria natural e os florívoros da espécie vegetal, passando por mudanças induzidas por florivoria sobre os atrativos visuais e químicos e sobre o néctar, até a realização de testes experimentais para investigar se as mudanças florais induzidas pela florivoria desencorajam a polinização por beija-flores.

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CAPÍTULO 1

DESVENDANDO OS MODULADORES ECOLÓGICOS DA FLORIVORIA EM ESCALA DE COMUNIDADE

DESVENDANDO OS MODULADORES ECOLÓGICOS DA FLORIVORIA EM ESCALA DE COMUNIDADE

RESUMO

A evolução e a radiação das Angiospermas e dos diversos grupos de insetos ocorreram e ainda ocorrem entrelaçadas. Os florívoros podem afetar o sucesso reprodutivo das plantas, alimentando-se de verticilos florais, alterando a forma da flor e afetando potencialmente a atração e visitação de polinizadores. Diversos fatores poderiam influenciar a quantidade de florivoria sofrida por uma espécie vegetal, como a concentração de recursos em escalas temporal e espacial, o que, seguindo a hipótese de concentração de recursos, levaria as espécies vegetais com distribuição espacial de indivíduos e de unidades de atração agrupadas a sofrer danos mais intensos devido à florivoria. Por outro lado, é possível que as flores sejam dispostas em inflorescências, podendo aumentar a exposição floral e, assim, influenciar os níveis de florivoria sofridos por tais flores. Entretanto, também é possível que, em um contexto de comunidade, níveis mais altos de florivoria ocorram em espécies que estão intimamente relacionadas filogenicamente, uma vez que espécies intimamente relacionadas podem apresentar características atrativas e defensivas semelhantes. Entretanto, o possível papel dos inimigos naturais das plantas, como os florívoros, na evolução dos caractéres florais tem sido amplamente desconsiderado. Somente recentemente foi proposta uma primeira tentativa de sistematizar padrões globais de como os caractéres florais podem afetar os danos florais e compreender a dinâmica eco-evolutiva das interações florívoro-flor. Entretanto, existe uma lacuna substancial no conhecimento em termos de estudos em escala de comunidade. Por isso, exploramos possíveis fatores ecológicos que poderiam atuar como moduladores da florivoria em uma comunidade de savana. Nosso objetivo é investigar a nível de comunidade se (i) a distribuição espacial das espécies vegetais, (ii) a distribuição espacial das unidades de atração dos polinizadores, (iii) o tamanho da unidade de atração dos polinizadores, (iv) o tipo de unidade de atração dos polinizadores, e (v) a relação filogenética são os moduladores da florivoria. Verificamos que 53,66% das espécies vegetais amostradas apresentaram índices de florivoria (IF) iguais a zero; 39,02% apresentaram $0,001 < IF < 1$; e as espécies vegetais restantes foram divididas entre as categorias superiores de IF, exceto $IF > 4,001$, que não ocorreu. Verificamos que a ocorrência de florivoria não é modulada pela distribuição espacial das espécies vegetais,

nem pela distribuição espacial das unidades de atração dos polinizadores, nem pelo tamanho da unidade de atração ($p > 0,05$), mas foi modulada pelo tipo de unidade de atração dos polinizadores apresentado por cada espécie vegetal ($p < 0,05$). Finalmente, observamos que o IF de cada espécie vegetal não apresenta sinal filogenético ($p > 0,05$). Nossos resultados sugerem que vários mecanismos de escape podem atuar na prevenção de uma alta incidência de florívoros em uma comunidade de savana. Levantamos algumas hipóteses para a ocorrência de baixos níveis de florivoria em escala de comunidade: (i) flores de várias espécies vegetais escapam da ação dos florívoros porque são pequenas e não são facilmente localizadas por eles; (ii) caracteres florais que atuam na comunicação planta-animal, podem atuar mais efetivamente na sinalização das flores aos polinizadores do que aos florívoros; (iii) flores de diferentes espécies vegetais têm características químicas que podem atuar como repelentes ou tornar os tecidos impalpáveis. É provável que vários outros fatores atuem em sistemas naturais envolvendo florívoros, e que estas hipóteses sejam interdependentes.

Palavras-chave: herbivoria floral, florívoro, escape fenológico, filogenia, polinização.

INTRODUÇÃO

Os processos de evolução e radiação das Angiospermas e dos diversos grupos de insetos ocorreram e ainda ocorrem entrelaçados (Labandeira, 2001; Labandeira, 2002). Os animais usam uma diversidade de características florais como pistas para localizar flores no ambiente (Schaefer e Ruxton, 2011; Willmer, 2011) e tais características podem ser moduladas por interações com florívoros (consumidores de flores) e polinizadores (Andrews et al., 2007). Florívoros podem afetar diretamente o sucesso reprodutivo das plantas, alimentando-se de estruturas reprodutivas (por exemplo, óvulos e pólen); ou indiretamente, alimentando-se de verticilos florais estéreis, alterando a forma das flores e potencialmente afetando a atração e visitação de polinizadores (McCall e Irwin, 2006 e referências).

Há um crescente corpo de literatura que tem se concentrado nos efeitos da florivoria sobre o sucesso reprodutivo de uma ou poucas espécies de plantas (Haas e Lortie, 2020 e referências). Esses estudos revelaram resultados variados, incluindo efeitos negativos (McCall, 2008; Kessler e Halitschke, 2009; Tsuji et al., 2016), neutros (Tunes et al., em preparação, Capítulo 5 desta tese) e até mesmo efeitos positivos da florivoria sobre sucesso reprodutivo das plantas em alguns cenários específicos (Botto-Mahan et al., 2011).

Além disso, outros fatores que podem influenciar a quantidade de florivoria sofrida por uma espécie de planta são a concentração de recursos em escalas temporal e espacial. De acordo com a hipótese de concentração de recursos (Root, 1973), insetos herbívoros especialistas deveriam ser mais abundantes em maiores concentrações de plantas hospedeiras em um mesmo local e tempo. Nesse cenário, poderíamos esperar que espécies vegetais com distribuição espacial de unidades de atração agrupada sofressem danos mais intensos devido à florivoria. A concentração do mesmo recurso trófico em um determinado lugar e tempo, por exemplo, muitos indivíduos de uma determinada espécie de planta florescendo ao mesmo tempo, pode levar a um escape fenológico pelas plantas de seus antagonistas (Augspurger, 1981), neste caso de florívoros, diluindo os efeitos negativos da florivoria entre todas as plantas. Por outro lado, é possível que espécies vegetais com unidades de atração de polinizadores maiores e mais abundantes (sensu Ramirez et al., 1990) atraiam mais florívoros devido a seu maior anúncio floral, da mesma forma que o anúncio floral atua em diferentes grupos de polinizadores (Bell, 1985; Conner e Rush, 1996; Andersson, 1988; Willmer, 2011). Por outro lado, é possível que as flores dispostas em inflorescências também possam aumentar seu anúncio floral e, assim,

influenciar os níveis de florivoria sofridos por tais flores (Boaventura et al., 2021). No entanto, também é possível que, em um contexto de comunidade, maiores níveis de florivoria ocorram em espécies que estão intimamente relacionadas filogeneticamente, uma vez que espécies intimamente relacionadas podem apresentar características semelhantes que atraem florívoros, ou, ao contrário, podem apresentar defesas semelhantes, como observado por Agrawal et al. (2009) para algumas características defensivas de plantas. Neste último caso, espécies vegetais filogeneticamente próximas apresentariam menos danos causados por florívoros ou apresentariam níveis mais baixos de dano por flor.

No entanto, a diversificação e evolução dos caractéres florais tem sido amplamente atribuída à seleção mediada por polinizadores, com apenas poucos estudos considerando o possível papel dos inimigos naturais das plantas na evolução dos caractéres florais (Galen, 1999; Moreira et al., 2019; Ramos e Schiestl, 2019). Além disso, no presente ano, Xiao et al. (2021) mostram que a florivoria em uma espécie vegetal do Cretáceo é indistinguível daquela de suas homólogas modernas, o que pode indicar que essa interação está em considerável equilíbrio com a aptidão da planta em uma escala evolutiva.

Embora a florivoria possa influenciar a reprodução das plantas e ser uma pressão seletiva significativa em relação à evolução dos caractéres florais, há uma lacuna substancial de pesquisas sobre a ocorrência da florivoria em sistemas naturais, seus moduladores ambientais e ecológicos e seus efeitos sobre a polinização e reprodução das plantas (Haas e Lortie, 2020). Boaventura et al. (2021) propuseram uma primeira tentativa de sistematizar padrões globais de como os caractéres florais podem afetar os danos florais e entender a dinâmica ecoevolutiva das interações florívoro-flor. No entanto, essa lacuna de conhecimento é ainda mais pronunciada em termos de estudos em escala de comunidade, que, até onde sabemos, nunca foram feitos. Portanto, neste estudo, exploramos possíveis fatores ecológicos que podem atuar como moduladores da florivoria em uma comunidade de savana. Nosso objetivo é investigar se (i) a distribuição espacial das espécies vegetais, (ii) a distribuição espacial das unidades de atração dos polinizadores, (iii) o tamanho da unidade de atração dos polinizadores, (iv) o tipo de unidade de atração dos polinizadores, e (v) a relação filogenética são os moduladores da florivoria.

MATERIAL E MÉTODOS

Realizamos este estudo em uma área de savana de aproximadamente 40 ha, localizada no município de Botucatu, estado de São Paulo, Brasil (22°54'21" - 22°55'05"S e 48°30'19" - 48°30' 09"O). O clima da região é classificado como Cfa, segundo Köppen, com verões quentes e chuvosos e invernos secos (Cunha e Martins, 2009).

Nesta área, estabelecemos 10 transecções de 100 metros de comprimento por 3 metros de largura (300 m² cada), com 100 m entre as transecções e pelo menos 50 m de distância de estradas e cercas, totalizando 3000 m² de área amostrada. Em 2017, amostramos todos as transecções em três momentos, no final da estação chuvosa (março e abril), durante a estação seca (julho e agosto) e no início da estação chuvosa (outubro e novembro). Consideramos como plantas individuais cada rameta que cresceu pelo a menos cinco centímetros de distância de outro rameta. Do ponto de vista ecológico, os rametas podem ser considerados indivíduos diferentes, embora compartilhem o mesmo genoma (Allaby, 2010). Avaliamos a ocorrência de florivoria em todos os indivíduos floridos enraizados dentro das trnasecções e correspondentes às espécies polinizadas por animais. Identificamos as espécies vegetais amostradas diretamente no campo, ou usando fotografias e exemplars de coleções de herbário, e para algumas espécies com a ajuda de especialistas. Foram depositados espécimes testemunho das espécies amostradas no Herbário Irina Delanova Gemtchujnicov (BOTU), IBB, UNESP (Apêndice 1).

Após a identificação das espécies vegetais, classificamos cada uma delas de acordo com seu tipo de unidade de atração de polinizadores (flores, intermediárias ou inflorescências), com base na classificação proposta por Ramirez et al. (1990). Para as espécies que apresentaram unidades de atração classificadas como intermediárias, utilizamos as flores como unidades para avaliação da florivoria. Amostramos até 30 unidades de atração por planta para cada espécie vegetal. Nos indivíduos com mais de 30 unidades de atração, amostramos as 30 unidades de atração mais ao sul (ponto cardeal padrão sorteado previamente à amostragem).

Avaliamos a porcentagem de área floral removida em cada unidade de atração e as categorizamos em um dos seis níveis de florivoria: nível 0 (0 a 0,9% de tecido floral removido), nível 1 (1 a 6%), nível 2 (6,1 a 12%), nível 3 (12,1 a 25%), nível 4 (25,1 a 50%) e nível 5 (> 50%), que foram adaptados da classificação proposta por Dirzo e Dominguez (1995) para herbivoria foliar. A partir do número de unidades de atração de cada espécie em cada categoria, calculamos o índice de florivoria (IF), de acordo com a equação $IF = (\Sigma ni\ xi)/N$, em que n é o número de unidades de atração com um determinado nível de florivoria, i é o número da categoria (0 a 5) e N é o número total de

unidades de atração amostradas na espécie. Além disso, foi calculado o índice de agregação de Morisita $IM = Q\frac{\sum_{K=1}^{Q} n_k(n_k-1)}{N(N-1)}$, em que Q é o total de transecções, *nk* é o número de indivíduos na k-ésima transecção e *N* é o número total de indivíduos (Morisita, 1959) de cada espécie. Nós consideramos que uma espécie apresentou distribuição espacial aleatória quando o IM estava próximo de 1, enquanto espécies com IM maior que 1 apresentaram distribuição agrupada, e espécies com IM menor que 1 apresentaram distribuição dispersa (Kanevski, 2004). Consideramos raras as espécies cujos indivíduos foram amostrados apenas uma vez. Além disso, adaptamos o índice de agregação de Morisita (Morisita, 1959) para calcular um índice de distribuição espacial de unidades de atração (IDEUA). Para isso, usamos a equação: $IDEUA = Q\frac{\sum_{K=1}^{Q} n_k(n_k-1)}{N(N-1)}$, em que Q é o total de transecções, *nk* é o número de unidades de atração na k-ésima transecção, e *N* é o número total de unidades de atração. Consideramos que as unidades de atração de polinizadores de uma espécie apresentam distribuição espacial aleatória quando IDEUA estava próximo de 1, enquanto espécies com IDEUA maior que 1 apresentam distribuição agrupada de unidades de atração de polinizadores e espécies com IDEUA menor que 1 apresentam distribuição dispersa de unidades de atração de polinizadores. Adicionalmente, classificamos as unidades de atração de cada espécie vegetal de acordo com seu tamanho em quatro categorias: "P", unidades de atração menores que 2 cm^3; "M", unidades de atração entre 4 cm^3 e 8 cm^3; "G", unidades de atração entre 10 cm^3 e 15 cm^3; e "EG", unidades de atração maiores que 20 cm^3. Realizamos essa classificação com base em informações disponíveis na Flora do Brasil (2020) e em medições de vouchers de herbário disponíveis no banco de dados speciesLink.

Ajustamos modelos lineares (MLs) para testar se a ocorrência de florivoria é afetada pela distribuição espacial das espécies vegetais, pela distribuição espacial das unidades de atração de polinizadores, pelo tamanho das unidades de atração e pelo tipo de unidade de atração de polinizadores apresentada por cada espécie vegetal. Quando necessário, aplicamos testes de contraste para comparações par-a-par. Além disso, construímos uma árvore filogenética das espécies amostradas com o PhyloMaker (Qian e Jin, 2016) e calculamos o sinal filogenético K de Blomberg para o índice de florivoria obtido para cada espécie. Realizamos todas as análises estatísticas e representações gráficas no ambiente computacional R (R Core Team, 2020), usando pacotes padrão e

adicionais beeswarm (Eklund, 2016), emmeans (Lenth, 2021), ggplot2 (Wickham, 2016), picante (Kembel et al., 2010) e phytools (Revell, 2012).

RESULTADOS

Amostramos 1230 plantas, de 82 espécies, pertencentes a 24 famílias (Tabela 1). As famílias com mais espécies foram Leguminosae, com 18 espécies, seguida de Asteraceae, com 15 espécies. Entre as espécies amostradas, 53,66% apresentaram índices de florivoria (IF) iguais a zero; 39,02% apresentaram $0,001 < IF < 1$; 4,88% das espécies apresentaram $1,001 < IF < 2$; 1,22% apresentou $2,001 < IF < 3$ e $3,001 < IF < 4$; nenhuma espécie vegetal apresentou $IF > 4,001$. As espécies com maiores índices de florivoria foram *Eriope crassipes* (IF = 4), *Solanum lycocarpum* (IF = 2,25), *Campomanesia adamantium* (IF = 1,6) e *Erythroxylum suberosum* (IF = 1,5).

Verificamos que a ocorrência de florivoria não é modulada pela distribuição espacial das espécies vegetais ($F_{[2, 79]} = 0,9383$; $p = 0,3956$; Figura 1A), nem pela distribuição espacial das unidades de atração dos polinizadores ($F_{[1, 80]} = 2,0946$; $p = 0,1517$; Figura 1B). Além disso, o tamanho da unidade de atração não afeta a florivoria sofrida por uma determinada espécie vegetal ($F_{[3, 78]} = 1,3129$; $p = 0, 2762$; Figura 1C). Por outro lado, verificamos que a ocorrência de florivoria foi de fato modulada pelo tipo de unidade de atração de polinizador apresentada por cada espécie vegetal ($F_{[2, 79]} = 3,3817$; $p = 0,03898$; Figura 1D; ver Anexo 2 para detalhes estatísticos).

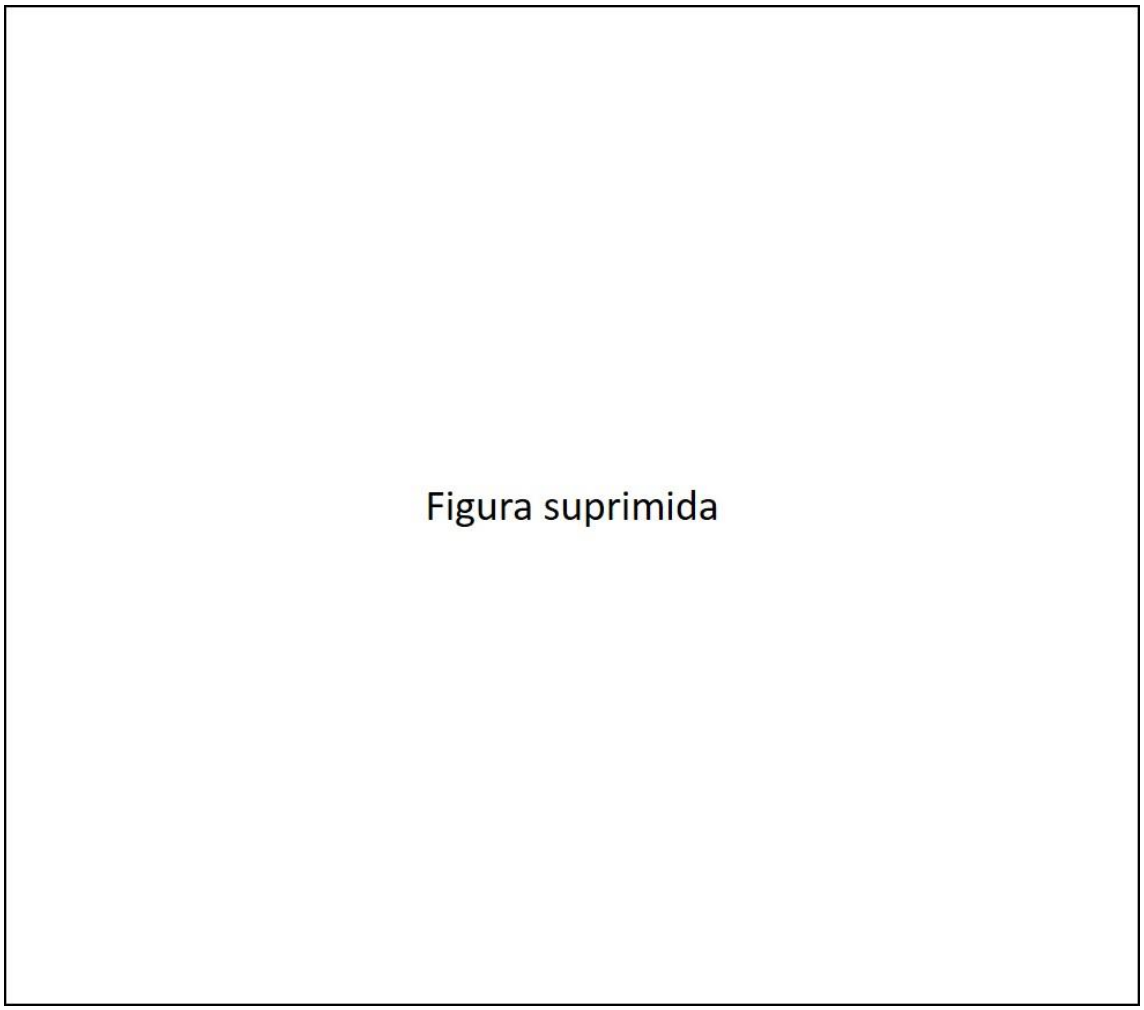

Figura 1. A. Distribuição espacial do índice de florivoria (IF) por espécie com base no índice de Morisita. B. Índice de florivoria (IF) por índice de distribuição espacial das unidades de atração (IDEUA). C. Índice de florivoria (IF) por tamanho da unidade de atração. D. Índice de florivoria (IF) por tipo de unidade de atração.

Tabela 1. Espécies de plantas floridas nas transecções amostradas, índice de florivoria (IF), distribuição espacial (DE) com base no índice de Morisita, índice de distribuição espacial de unidades de atração (IDEUA), adaptado do índice de Morisita, tipo de unidade de atração (UA), com base em Ramirez et al. (1990) e tamanho da unidade de atração (Tamanho). P < 2 cm^3, M: 4 – 8 cm^3, G: 10 – 15 cm^3, EG > 20 cm^3.

Espécies	IF	DE	IDEUA	UA	Tamanho
Acanthaceae					
Ruelia geminiflora Kunth	0.500	Dispersa	1.667	Flor	EG
Annonaceae					
Duguetia furfuracea (A.St.-Hil.) Saff.	0.000	Rara	0.000	Flor	G
Asteraceae					

Acanthospermum australe (Loefl.) Kuntze	0.000	Agrupada	4.667	Inflorescência	P
Achyrocline alata (Kunth) DC	0.000	Dispersa	5.238	Inflorescência	M
Bidens gardneri Baker	0.423	Agrupada	2.881	Inflorescência	M
Campuloclinium macrocephalum (Less.) DC.	0.000	Rara	10.000	Inflorescência	M
Chaptalia integerrima (Vell.) Burkart	0.000	Dispersa	0.000	Inflorescência	M
Chromolaena maximiliani (Schrad. ex DC.) R.M.King & H.Rob.	0.028	Agrupada	3.489	Inflorescência	P
Chromolaena sp.	0.000	Agrupada	5.239	Inflorescência	P
Chromolaena squalida (DC.) R.M.King & H.Rob.	0.000	Agrupada	2.601	Inflorescência	P
Elephantopus mollis Kunth	0.000	Agrupada	3.478	Inflorescência	P
Emilia fosbergii Nicolson	0.000	Agrupada	3.211	Inflorescência	P
Lessingianthus argyrophyllus (Less.) H.Rob.	0.000	Agrupada	10.000	Inflorescência	G
Lessingianthus bardanoides (Less.) H.Rob.	0.029	Agrupada	10.000	Inflorescência	EG
Lessingianthus sp.	0.000	Agrupada	3.182	Inflorescência	EG
Orthopappus angustifolius (Sw.) Gleason	0.000	Agrupada	4.487	Inflorescência	M
Vernonanthura macronulata (Less.) H.Rob.	0.000	Agrupada	4.022	Inflorescência	M
Bignoniaceae					
Zeyheria montana Mart.	0.182	Agrupada	3.308	Flor	G
Celastraceae					
Peritassa campestris (Cambess.) A.C. Sm.	0.476	Agrupada	10.000	Flor	P
Tontelea micrantha (Mart.) A.C. Sm.	0.000	Rara	10.000	Flor	P
Commelinaceae					
Commelina erecta L.	0.000	Agrupada	4.444	Flor	P
Convolvulaceae					
Distimake digitatus (Spreng.) A.R. Simões & Staples	0.714	Agrupada	2.381	Flor	EG
Ebenaceae					
Diospyros lasiocalyx (Mart.) B.Walln.	0.000	Agrupada	5.997	Flor	P
Erythroxylaceae					
Erythroxylum campestre A.St.-Hil.	0.400	Agrupada	4.667	Flor	P
Erythroxylum suberosum A.St.-Hil.	1.500	Rara	10.000	Flor	P
Erythroxylum tortuosum Mart.	0.667	Rara	10.000	Flor	P
Euphorbiaceae					
Croton campestris A.St.-Hil.	0.000	Rara	10.000	Flor	P

Croton glandulosus L.	0.000	Agrupada	2.420	Flor	P
Lamiaceae					
Eriope crassipes Benth.	4.000	Rara	0.000	Flor	P
Hyptis radicans (Pohl) Harley & J.F.B.Pastore	0.000	Dispersa	4.872	Intermediária	M
Salvia minarum Briq.	0.056	Agrupada	3.268	Flor	P
Salvia sp.	0.000	Dispersa	2.000	Flor	P
Leguminosae					
Andira humilis Mart. ex Benth.	1.012	Agrupada	5.398	Flor	P
Chamaecrista flexuosa (L.) Greene	0.000	Rara	0.000	Flor	P
Chamaecrista sp. 1	0.500	Dispersa	2.222	Flor	P
Chamaecrista sp. 2	0.000	Agrupada	4.667	Flor	P
Chamaecrista sp. 3	0.000	Rara	10.000	Flor	P
Chamaecrista sp. 4	0.727	Agrupada	10.000	Flor	P
Chamaecrista sp. 5	0.000	Agrupada	10.000	Flor	P
Chamaecrista sp. 6	0.000	Rara	10.000	Flor	P
Crotalaria sp.	0.205	Agrupada	10.000	Flor	P
Desmodium adscendens (Sw.) DC.	0.000	Agrupada	7.143	Flor	P
Desmodium incanum (Sw.) DC.	0.095	Agrupada	4.611	Flor	P
Mimosa pudica L.	0.000	Rara	10.000	Inflorescência	P
Mimosa sp. 1	0.000	Rara	10.000	Inflorescência	P
Mimosa sp. 2	0.000	Rara	10.000	Inflorescência	P
Mimosa xanthocentra Mart.	0.000	Dispersa	2.372	Inflorescência	P
Stryphnodendron adstringens (Mart.) Coville	0.000	Agrupada	7.949	Inflorescência	G
Stylosanthes sp.	0.000	Rara	10.000	Flor	P
Zornia latifolia Sm.	0.000	Agrupada	2.500	Flor	P
Lythraceae					
Cuphea calophylla Cham. & Schltdl.	0.804	Agrupada	2.965	Flor	P
Cuphea micrantha Kunth	0.679	Agrupada	2.908	Flor	P
Cuphea sp.	0.000	Dispersa	0.000	Flor	P
Cuphea thymoides Cham. & Schltdl.	1.127	Agrupada	1.541	Flor	P
Malpighiaceae					
Byrsonima coccolobifolia Kunth	0.361	Agrupada	4.652	Flor	P
Byrsonima intermedia A.Juss.	0.070	Agrupada	5.502	Flor	P
Byrsonima sp.	0.050	Rara	10.000	Flor	P
Galphimia australis Chodat	1.000	Agrupada	10.000	Flor	P
Malvaceae					

Peltaea polymorpha (A.St.-Hil.) Krapov. & Cristóbal	1.000	Agrupada	2.000	Flor	EG
Sida cordifolia L.	0.500	Agrupada	3.333	Flor	P
Sida glaziovii K.Schum.	0.322	Agrupada	3.999	Flor	P
Sida linearifolia A.St.-Hil.	0.333	Agrupada	2.190	Flor	P
Waltheria communis A.St.-Hil.	1.000	Dispersa	4.444	Intermediária	M
Waltheria indica L.	0.000	Agrupada	5.714	Intermediária	P
Melastomataceae					
Miconia sp.	0.300	Rara	10.000	Flor	P
Pleroma stenocarpum (Schrank et Mart. ex DC.) Triana	0.568	Agrupada	9.200	Flor	EG
Myrsinaceae					
Myrsine guianensis (Aubl.) Kuntze	0.000	Agrupada	10.000	Flor	P
Myrtaceae					
Campomanesia adamantium (Cambess.) O.Berg	1.600	Dispersa	6.000	Flor	P
Ochnaceae					
Ouratea spectabilis (Mart.) Engl.	0.000	Rara	0.000	Flor	M
Oxalidaceae					
Oxalis conorrhiza Jacq.	0.222	Agrupada	5.000	Flor	P
Rubiaceae					
Borreria latifolia (Aubl.) K.Schum.	0.000	Agrupada	10.000	Flor	P
Borreria poaya (A.St.-Hil.) DC.	0.000	Agrupada	8.667	Flor	P
Borreria tenella (Kunth) Cham. & Schltdl.	0.043	Agrupada	2.727	Inflorescência	P
Hexasepalum teres (Walter) J.H.Kirkbr.	0.008	Agrupada	2.192	Flor	P
Mitracarpus hirtus (L.) DC.	0.000	Agrupada	2.500	Intermediária	M
Richardia brasiliensis Gomes	0.000	Rara	10.000	Flor	P
Richardia grandiflora (Cham. & Schltdl.) Steud.	0.467	Agrupada	1.997	Flor	P
Sapotaceae					
Chrysophyllum marginatum (Hook. & Arn.) Radlk.	0.000	Rara	10.000	Flor	P
Solanaceae					
Schwenckia americana Rooyen ex L.	0.000	Agrupada	3.333	Flor	P
Solanum lycocarpum A.St.-Hil.	2.250	Dispersa	3.333	Flor	EG
Verbenaceae					
Lippia lupulina Cham.	0.000	Dispersa	3.609	Intermediária	EG
Stachytarpheta cayennensis (Rich.) Vahl	0.127	Agrupada	5.530	Flor	P

Por fim, observamos que o índice de florivoria de cada espécie vegetal não apresenta sinal filogenético (K = 0,3308; p = 0,087; Figura 2).

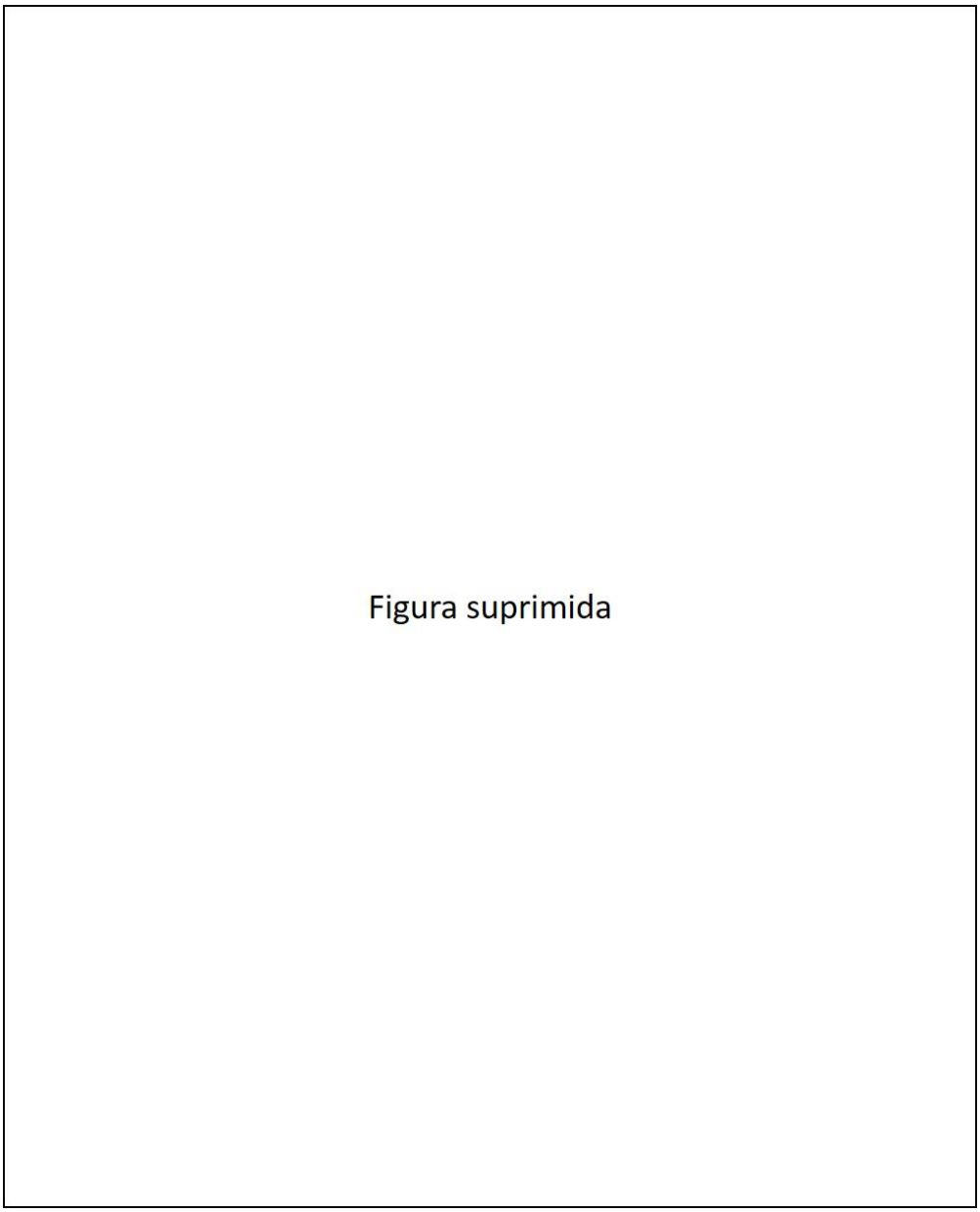

Figura 2. Árvore filogenética das espécies vegetais amostradas em vegetação de savana, criada com base em Quian e Jin (2016), incluindo o índice de florivoria (IF) calculado para cada espécie vegetal.

DISCUSSÃO

Os resultados obtidos em nosso estudo revelaram que, em uma comunidade de cerrado, a maioria das espécies vegetais apresenta níveis de florivoria muito baixos, com mais de 90% das espécies apresentando índice de florivoria variando de 0 a 1%. Mais da

metade das espécies amostradas apresentaram índices de florivoria iguais a zero, e outros 37,04% apresentaram índices baixos, entre 0,001 e 1%. No geral, os danos observados nas espécies vegetais avaliadas foram pequenos, sugerindo que a maioria dos danos às flores foi causada por invertebrados, que são conhecidos por remover apenas pequenas porções da corola a cada mordida (Leavitt e Robertson, 2006). A ocorrência de pequenos danos observados na maioria das espécies vegetais pode ser devido à presença de compostos defensivos que constituem os tecidos florais que podem levar à sua baixa palatabilidade (Howe e Jander, 2008), ou ainda a compostos voláteis repelentes, que podem fazer parte do odor floral ou ser liberados como resposta após o dano (Kessler e Halitschke, 2009 e suas referências). Assim, a presença desses compostos repelentes não palatáveis ou voláteis poderia levar os florívoros a interromper sua alimentação e abandonar a planta, resultando apenas na presença de pequenas marcas.

É interessante notar que as espécies com os maiores índices de florivoria *Eriope crassipes* (4), *Solanum lycocarpum* (2,25), *Erythroxylum suberosum* (1,5) e *Campomanesia adamantium* (1,2) são polinizadas por abelhas pequenas, abelhas grandes, e duas são generalistas, respectivamente. Ao contrário do esperado, nossos resultados, em geral, mostram que não há relação entre o sistema de polinização da espécie e seu índice de florivoria.

Além disso, 42,22% das espécies que apresentaram índice de florivoria igual a zero apresentaram abundância relativa superior à média da comunidade, e as espécies vegetais com índice de florivoria maior apresentaram baixa abundância relativa. Esse resultado sugere que espécies menos abundantes estão mais sujeitas à ação voraz de antagonistas, enquanto espécies mais abundantes com o mesmo recurso podem escapar da ação de florívoros, como no caso de antagonistas predadores de sementes que infestam a planta durante a floração (Augspurger, 1981). No entanto, é interessante notar que 57,78% das espécies que apresentaram índice de florivoria igual a zero foram pouco abundantes. Esse resultado indica que existem outros fatores que podem influenciar a quantidade de florivoria que afeta uma determinada espécie vegetal, como o escape espacial devido ao tamanho da planta, como proposto para herbívoros foliares (Del-Claro e Torezan-Silingardi, 2012). Por outro lado, o fato de verificarmos que o tipo de unidade de atração, ou seja, como as flores estão dispostas dentro de uma planta, modula a intensidade em que ocorre a florivoria, é semelhante às estratégias de escape observadas para a herbivoria foliar. Podemos estabelecer um paralelo com a "hipótese da aparência de plantas" (Feeny, 1976), que postula que espécies de plantas conspícuas apresentam

maior probabilidade de serem encontradas por herbívoros e, portanto, elas teriam evoluído sob forte pressão seletiva de herbívoros, o que poderia ter levado à evolução de maiores concentrações de defesas químicas e físicas contra herbívoros. Portanto, é possível que plantas que apresentam inflorescências como unidades de atração de polinizadores sejam mais defendidas contra os florívoros. Boaventura et al. (2021) sugerem que a 'hipótese da aparência da planta' pode se aplicar inversamente a flores solitárias, que apresentariam menor probabilidade de serem encontradas por florívoros na natureza, e não teriam evoluído sob pressão tão forte deles, não tendo fortes defesas selecionadas ao longo do tempo. No entanto, argumentamos que é possível que, para entender completamente as implicações desta hipótese na florivoria, devamos avaliar o anúncio floral e a conspicuidade da planta como uma combinação do tipo e tamanho da unidade de atração. Além disso, a comunidade amostrada é predominantemente composta por espécies herbáceas, cuja maioria apresentou índice florivoria igual a zero (80,00%). Conforme apontado por Wiklund e Friberg (2009), plantas diminutas podem escapar da ação dos antagonistas porque não são facilmente localizadas por eles. Portanto, é possível que em comunidades de cerrado, onde as espécies herbáceas são abundantes, haja pouca florivoria em geral.

Além disso, no caso de plantas diminutas com flores de tamanho reduzido, o florívoro pode gastar muita energia e tempo para obter poucos recursos, fazendo com que esses animais não invistam na busca de tais flores, pois o tempo gasto na busca dessas flores provavelmente não será compensado pela quantidade de recurso obtido, conforme os preceitos da teoria do forrageamento ótimo (MacArthur e Pianka, 1966). Assim, seria de se esperar que plantas com unidades de atração menores sofressem níveis mais baixos de florivoria, uma vez que suas unidades de atração seriam menos conspícuas no meio. Além disso, alguns herbívoros foliares preferem folhas maiores (Auerbach e Simberloff, 1989), de modo que se espera que os florívoros prefiram flores maiores, assim como os polinizadores (Bell, 1985; Conner e Rush, 1996; Andersson, 1988; Martin, 2004). Neste caso, flores maiores apresentariam maior incidência de florivoria, por preferência por parte dos florívoros ou por estimularem uma maior frequência de visitas de florívoros, como observado por Thomson (2001) para Lepidoptera, levando a uma maior incidência de florivoria nas flores. No entanto, nossos resultados contrariam essas expectativas, revelando que não há relação entre o tamanho das unidades de atração de uma determinada espécie vegetal e a intensidade da florivoria que ela sofre.

Por outro lado, em espécies que ocorrem agrupadas, é possível traçar um paralelo que, assim como os polinizadores podem formar imagens de busca e localizar flores de forma mais eficiente (Gegear e Laverty, 2001), os florívoros também são capazes de fazê-lo, bem como vários grupos de herbívoros foliares (Brown et al., 1991 e suas referências). Nesse caso, o recurso obtido pelos florívoros possivelmente ultrapassaria o gasto energético da busca pelo recurso, fazendo com que os florívoros se alimentassem vorazmente dos indivíduos da espécie em questão. No entanto, ainda existem muitas questões não respondidas sobre os mecanismos subjacentes envolvidos em como os herbívoros escolhem os manchas em que irão forragear, sendo crucial ter uma compreensão abrangente do modo de busca dos herbívoros (Hambäck e Englund, 2005). Além disso, a maioria dos insetos herbívoros são especializados na obtenção de alimentos em um ou poucos gêneros ou em uma única família de plantas e apenas 10% se alimentam de plantas de mais de três famílias distintas (Bernays e Graham, 1988). Portanto, esperávamos que a ação dos florívoros sobre um gênero ou família pudesse ter selecionado estratégias de defesa semelhantes em espécies mais próximas, fazendo com que essas espécies fossem afetadas por florívoros em níveis semelhantes. No entanto, nosso estudo revelou que não há relação entre a incidência de florivoria e o parentesco filogenético entre as espécies na comunidade.

O presente estudo é, até onde sabemos, o primeiro a estudar a interação da florivoria em escala de comunidade buscando elucidar quais processos ecológicos e variáveis biológicas podem modular a intensidade dessa interação em comunidades naturais. Além disso, este estudo destaca o fato de que os sistemas florívoro-flor-polinizador podem ser essencialmente espécie-específicos, uma vez que a florivoria sofrida por uma espécie de planta não pode ser prevista com base em seus polinizadores, sua abundância relativa, o tamanho de suas unidades de atração, nem seu parentesco filogenético, mas está relacionada ao tipo de unidade de atração apresentada por cada espécie vegetal. Com base nos resultados obtidos neste estudo, é possível levantar algumas hipóteses para a ocorrência de baixos níveis de florivoria em nível de comunidade: (i) flores de várias espécies de plantas escapam à ação dos florívoros por serem pequenas e de difícil localização por eles; (ii) caracteres florais que atuam na comunicação planta-animal, podem atuar mais efetivamente na sinalização de flores aos polinizadores do que aos florívoros; (iii) flores de diferentes espécies vegetais possuem características químicas que podem atuar como repelentes ou tornar os tecidos das flores

impalatáveis. É provável que vários outros fatores atuem em sistemas naturais envolvendo florívoros, e que essas hipóteses sejam interdependentes.

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Apêndice 1. Número dos vouchers do material testemunho das espécies amostradas neste estudo e depositadas no Herbário Irina Delanova Gemtchujnicov (BOTU), IBB, UNESP.

Espécies	BOTU
Acanthaceae	
Ruelia geminiflora Kunth	33167
Annonaceae	
Duguetia furfuracea (A.St.-Hil.) Saff.	33004
Asteraceae	
Acanthospermum australe (Loefl.) Kuntze	32879
Achyrocline alata (Kunth) DC	32864
Bidens gardneri Baker	32857
Campuloclinium macrocephalum (Less.) DC.	33024
Chaptalia integerrima (Vell.) Burkart	33030
Chromolaena maximiliani (Schrad. ex DC.) R.M.King & H.Rob.	33014
Chromolaena sp.	-
Chromolaena squalida (DC.) R.M.King & H.Rob.	33010
Elephantopus mollis Kunth	33029
Emilia fosbergii Nicolson	32873
Lessingianthus argyrophyllus (Less.) H.Rob.	-
Lessingianthus bardanoides (Less.) H.Rob.	33042
Lessingianthus sp.	-
Orthopappus angustifolius (Sw.) Gleason	32869
Vernonanthura macronulata (Less.) H.Rob.	33063
Bignoniaceae	
Zeyheria montana Mart.	33009
Celastraceae	
Peritassa campestris (Cambess.) A.C. Sm.	33055
Tontelea micrantha (Mart.) A.C. Sm.	33176
Commelinaceae	
Commelina erecta L.	-
Convolvulaceae	
Distimake digitatus (Spreng.) A.R. Simões & Staples	33016
Ebenaceae	
Diospyros lasiocalyx (Mart.) B.Walln.	-
Erythroxylaceae	
Erythroxylum campestre A.St.-Hil.	-
Erythroxylum suberosum A.St.-Hil.	-
Erythroxylum tortuosum Mart.	-
Euphorbiaceae	
Croton campestris A.St.-Hil.	-
Croton glandulosus L.	-
Lamiaceae	
Eriope crassipes Benth.	-

Hyptis radicans (Pohl) Harley & J.F.B.Pastore	32866
Salvia minarum Briq.	-
Salvia sp.	-
Leguminosae	-
Andira humilis Mart. ex Benth.	-
Chamaecrista flexuosa (L.) Greene	-
Chamaecrista sp. 1	-
Chamaecrista sp. 2	-
Chamaecrista sp. 3	-
Chamaecrista sp. 4	-
Chamaecrista sp. 5	-
Chamaecrista sp. 6	-
Crotalaria sp.	-
Desmodium adscendens (Sw.) DC.	33169
Desmodium incanum (Sw.) DC.	33184
Mimosa pudica L.	-
Mimosa sp. 1	-
Mimosa sp. 2	
Mimosa xanthocentra Mart.	33160
Stryphnodendron adstringens (Mart.) Coville	-
Stylosanthes sp.	33168
Zornia latifolia Sm.	-
Lythraceae	
Cuphea calophylla Cham. & Schltdl.	33006
Cuphea micrantha Kunth	33021
Cuphea sp.	-
Cuphea thymoides Cham. & Schltdl.	33032
Malpighiaceae	
Byrsonima coccolobifolia Kunth	33053
Byrsonima intermedia A.Juss.	-
Byrsonima sp.	-
Galphimia australis Chodat	33170
Malvaceae	
Peltaea polymorpha (A.St.-Hil.) Krapov. & Cristóbal	33051
Sida cordifolia L.	-
Sida glaziovii K.Schum.	33008
Sida linearifolia A.St.-Hil.	32872
Waltheria communis A.St.-Hil.	-
Waltheria indica L.	33007
Melastomataceae	
Miconia sp.	-
Pleroma stenocarpum (Schrank et Mart. ex DC.) Triana	33023/ 33052
Myrsinaceae	

Myrsine guianensis (Aubl.) Kuntze	33017
Myrtaceae	
Campomanesia adamantium (Cambess.) O.Berg	-
Ochnaceae	
Ouratea spectabilis (Mart.) Engl.	33043
Oxalidaceae	
Oxalis conorrhiza Jacq.	-
Rubiaceae	
Borreria latifolia (Aubl.) K.Schum.	-
Borreria poaya (A.St.-Hil.) DC.	33020/ 33049
Borreria tenella (Kunth) Cham. & Schltdl.	32877/ 33046
Hexasepalum teres (Walter) J.H.Kirkbr.	33028
Mitracarpus hirtus (L.) DC.	-
Richardia brasiliensis Gomes	-
Richardia grandiflora (Cham. & Schltdl.) Steud.	32856/ 33025
Sapotaceae	
Chrysophyllum marginatum (Hook. & Arn.) Radlk.	33033
Solanaceae	
Schwenckia americana Rooyen ex L.	-
Solanum lycocarpum A.St.-Hil.	33022
Verbenaceae	
Lippia lupulina Cham.	33054
Stachytarpheta cayennensis (Rich.) Vahl	33019

Apêndice 2. Estatísticas detalhadas das comparações par-a-par para avaliar se a ocorrência de florivoria foi de fato modulada pelo tipo de unidade de atração de polinizadores apresentada por cada espécie vegetal. Os resultados estatisticamente significativos estão marcados em negrito.

Comparação par-a-par	Estimativa	EP	gl	T	p
Flor - Inflorescência	0.383	0.149	79	2,5720	**0.0318**
Flor - Intermédiária	0.208	0.271	79	0.765	0.7256
Inflorescência - Intermédiária	-0.175	0.289	79	-0.605	0.8177

CAPÍTULO 2

ALTERAÇÕES NA SIMETRIA FLORAL E NA ÁREA FLORAL AFECTAM A ESCOLHA DAS ABELHAS?

ALTERAÇÕES NA SIMETRIA FLORAL E NA ÁREA FLORAL AFECTAM A ESCOLHA DAS ABELHAS?

RESUMO

Características florais que agem como atrativos para polinizadores também atraem herbívoros florais (florívoros), que por sua vez alteram características florais ao se alimentarem de flores, levando a possíveis mudanças no comportamento dos polinizadores. No entanto, a maioria dos estudos que exploraram os efeitos da florivoria no desempenho das plantas concentram-se apenas nas consequências da florivoria, negligenciando as vias de comunicação envolvidas na comunicação planta-polinizador que podem ser alteradas pela florivoria e como cada característica floral isolada pode desempenhar um papel na manutenção ou ruptura dessa via de comunicação. A atração dos polinizadores pelas flores é uma consequência de como os polinizadores percebem as complexas pistas florais exibidas por eles. Entre elas estão as pistas visuais, que são multicomponentes. As características visuais florais podem ser afetadas diretamente pelos florívoros que se alimentam de flores, pois geralmente removem porções das bordas da corola modificando a simetria da flor e reduzindo a área de sua superfície. Assim, as abelhas podem reagir às flores danificadas não reconhecendo o novo contorno floral assimétrico ou evitando essas flores reduzidas, pois selecionam preferencialmente flores maiores para forragear. Portanto, nosso objetivo foi testar se a florivoria afeta o comportamento das abelhas por meio de mudanças na simetria e área floral. Esperamos que mudanças nas pistas visuais afetem o comportamento das abelhas, pois os atrativos visuais são evidentes nas flores melitófilas. Para isso, foram utilizados quatro tipos de flores artificiais: (i) flores intactas (zigomorfas), (ii) flores com florivoria em apenas um lado (assimétricas), semelhante ao dano observado na espécie modelo (*Centrosema pubescens*) em condições naturais. Considerando que a frequência de visitas às flores do tipo (ii) pode ser influenciada tanto pela perda de zigomorfia quanto pela redução de área, utilizamos dois outros tipos florais para controlar essas duas variáveis: (iii) flores com florivoria em ambos os lados (zigomorfa), mantendo a área igual à área das flores do tipo (ii); e (iv) flores com padrão de florivoria assimétrico, semelhante ao das flores do tipo (ii), mas com área igual à área das flores do tipo (i). Utilizamos três tratamentos: (1) abelhas *naïve*, ou seja, abelhas que se alimentaram apenas de néctar sem nunca terem visitado uma flor natural ou artificial, (2) abelhas que foram treinadas para obter néctar de flores intactas, flores do tipo (i); e (3) abelhas treinadas para obter néctar de flores com

sinais de florivoria, flores do tipo (ii). Registramos todas as visitas feitas por cada abelha a cada tipo floral. Observamos que o comportamento das abelhas, em termos de número de visitas, porcentagem relativa de visitas e em termos da probabilidade de uma abelha realizar sua primeira visita a um tipo específico de flor, não foi afetado pelas mudanças na simetria floral promovidas pela florivoria, independentemente da experiência anterior das abelhas. Além disso, o número de visitas e a porcentagem relativa de visitas realizadas pelas abelhas não foram afetadas pela redução da área floral devido à florivoria, também independentemente da experiência anterior das abelhas. Por outro lado, as abelhas que forrageiam em flores com área floral reduzida apresentaram maior probabilidade de realizar sua primeira visita a uma flor com área floral padrão, independentemente do tipo de simetria apresentada pelas flores. Já as abelhas ingênuas e as abelhas que forrageavam em flores com área floral padrão realizaram suas primeiras visitas às flores com área floral padrão e reduzida indistintamente. Esses resultados indicam que as alterações na simetria floral e na área floral (individual ou concomitantemente), como as causadas pela ação dos florívoros, não afetam a comunicação planta-polinizador.

Palavras-chave: área floral, atrativos visuais, comunicação planta-polinizador, florivoria, polinização, simetria floral.

INTRODUÇÃO

A extensão em que o dano floral causado por florívoros pode alterar o comportamento dos polinizadores e afetar a aptidão das plantas tem sido mais profundamente explorado apenas recentemente (Liao et al., 2013; Missagia e Alves, 2017; Ye et al., 2017; Rusman et al., 2019; Capítulo 4 desta tese). No entanto, a maioria desses estudos se concentra apenas nas consequências da florivoria (Haas e Lortie, 2020), negligenciando as vias envolvidas na comunicação planta-polinizador que poderiam ter sido alteradas pela florivoria. Além disso, sabemos pouco sobre o papel que cada caractere floral isolado desempenha na manutenção ou ruptura dessas vias de comunicação.

A atração dos polinizadores pelas flores é consequência de como os polinizadores percebem as complexas pistas florais exibidas por elas (Leonard e Masek, 2014; Schaefer e Ruxton, 2015; Kantsa et al., 2017; Leonard e Francis, 2017). Entre elas estão as pistas visuais que são multicomponentes (Leonard et al., 2011). A atração visual dos polinizadores pelas flores pode estar relacionada à simetria da flor (Giurfa et al., 1996), contorno (Dafni e Kevan, 1997; Willmer, 2011), forma (Schiestl e Johnson, 2013), tamanho (Dafni e Kevan, 1997; Martin, 2004), cor e padrão de cor intrafloral (Papiorek et al., 2016).

Embora o interesse científico pelos sistemas visuais e preferências de diferentes grupos de polinizadores tenha crescido exponencialmente (eg. Tripp e Manos, 2008; Lunau et al., 2011; Papiorek et al., 2016), a importância da simetria floral para a interação com polinizadores ainda é pouco explorada. De fato, o contorno floral pode ser afetado diretamente pelos florívoros que se alimentam de flores, pois estes geralmente removem porções das bordas da corola (Cap. 4 desta tese) modificando a simetria da flor. Sabe-se que as abelhas preferem flores com simetria (Giurfa et al., 1996), seja com simetria radial ou bilateral voltada para baixo (Giurfa e Lehrer, 2001). No entanto, não se sabe como as abelhas respondem às novas formas florais que aparecem em uma planta em que estão se alimentando, como seria o caso de flores parcialmente consumidas por florívoros. Essas flores, além de perderem sua simetria, também têm sua área superficial reduzida. Portanto, as abelhas podem reagir a flores danificadas não reconhecendo o novo contorno floral assimétrico ou evitando essas flores com áreas reduzidas, já que selecionam preferencialmente flores maiores para forragear (Martin, 2004).

Além disso, estudos anteriores mostraram que as abelhas exibem certas preferências associadas a essas características florais, que podem ser modificadas pela

florivoria. Portanto, neste estudo, pretendemos testar especificamente se dois resultados da florivoria, a perda de simetria e a redução da área da superfície, afetam o comportamento das abelhas. Esperamos que mudanças nas pistas visuais afetem o comportamento das abelhas, pois os atrativos visuais são evidentes nas flores polinizadas por este grupo.

MATERIAL E MÉTODOS

Neste estudo, determinamos experimentalmente o papel de dois resultados associados à florivoria, mudanças na simetria floral e na área da superfície, no comportamento de visitas de abelhas. Utilizamos como modelo o contorno floral e o padrão de remoção da corola observado em condições naturais em *Centrosema pubescens* Benth. (Leguminosae), uma espécie de planta Neotropical polinizada por grandes abelhas pertencentes ao gênero *Bombus* (conforme relatado no Capítulo 3). Usamos o padrão de florivoria mais frequente determinado em campo nesta espécie de planta (Figura 1; ver Capítulo 3) para fazer as flores artificiais. O uso de flores artificiais evita a influência de outras variáveis, como variações naturais na cor da flor, tamanho e aroma emitido, garantindo que o efeito observado neste experimento seja devido apenas a alterações na simetria e na área da superfície das flores.

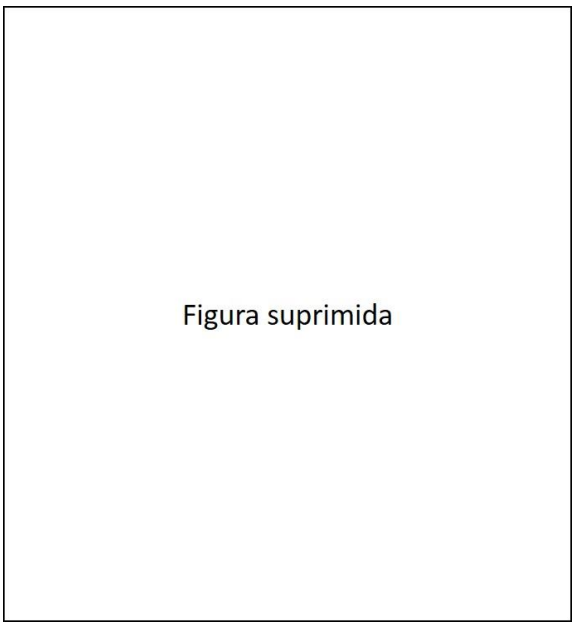

Figura 1. Flor de *Centrosema pubescens* Benth. (Leguminosae) mostrando seu padrão de florivoria mais frequente. Esta foi a flor modelo e o dano floral usado para fazer as flores artificiais.

Nós projetamos este experimento para que pudéssemos identificar se as abelhas tinham alguma preferência inata ou aprendida por flores intactas. Para isso, foram utilizados quatro tipos de flores artificiais (Figura 2): [i] flores intactas simétricas (representando o contorno original do modelo, apresentando zigomorfia), [ii] assimétricas (representando o padrão comum de florivoria em apenas um lado da flor. Considerando que a florivoria também pode afetar a superfície floral, utilizamos dois outros tipos florais para controlar essas duas variáveis: [iii] flores simétricas com florivoria em ambos os lados, mantendo a zigomorfia, mas com área da superfície reduzida (igual à área das flores do tipo [ii]); e [iv] flores assimétricas com o mesmo padrão florivoria das flores do tipo [ii], mas com área da superfície igual à área das flores do tipo [i] (Figura 2).

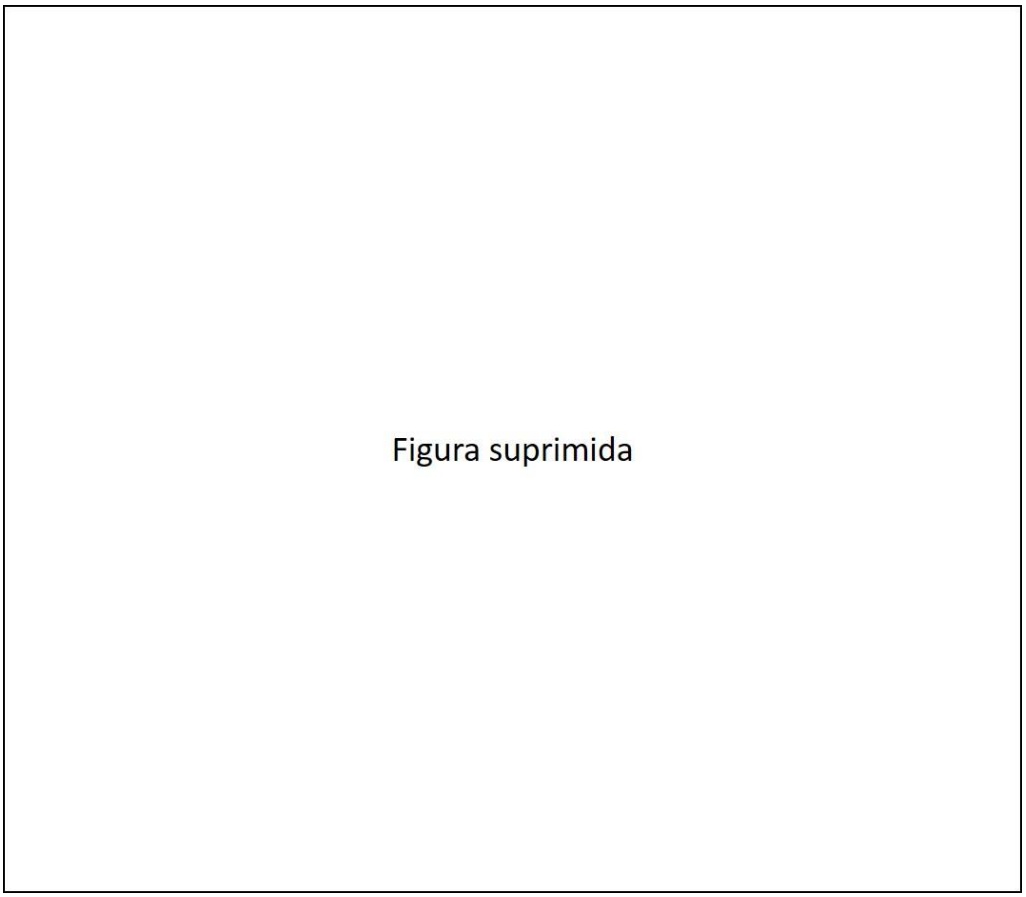

Figura 2. Tipos de flores usados no delineamento experimental para testar as consequências da florivoria, mudanças na simentria floral e na área da superfície floral, sobre o comportamento de visita das abelhas.

Todas as flores continham 30 µL de néctar artificial preparado com sacarose e água na proporção 1:1. Utilizamos colmeias comerciais de *Bombus terrestris* contendo inicialmente uma rainha e de 30 a 45 operárias. Foram aplicados três tratamentos: (1)

abelhas *naïves*, ou seja, abelhas que se alimentaram apenas de néctar sem nunca terem visitado uma flor natural ou artificial, (2) abelhas que foram treinadas para obter néctar de flores intactas, flores tipo [i]; e (3) abelhas treinadas para obter néctar de flores com sinais de florivoria, flores do tipo [ii]. Os turnos de treinamento foram realizados utilizando apenas flores do tipo [i] e do tipo [ii], pois esses são os cenários aos quais as abelhas estariam acostumadas na natureza, flores intactas e flores com florivoria. Flores do tipo [iii] e do tipo [iv] foram empregadas apenas como controles de área de superfície floral e simetria. O treinamento consistiu em cinco turnos consecutivos de visitas das abelhas a flores artificiais de um dos dois tipos [i] ou [ii]. As abelhas foram soltas uma a uma da colmeia, de modo que apenas uma abelha voava pela arena de cada vez. As abelhas voaram livremente e, depois de um tempo, visitaram as flores artificiais. Após a primeira rodada de visitas, marcamos as abelhas com tinta guache não tóxica de cores diferentes para que pudéssemos identificá-las após o retorno à colmeia. A abelha treinada retornava à colmeia após cada uma das cinco rodadas de treinamento para regurgitar o néctar coletado nos potes de néctar dentro da colmeia, sendo então liberada para realizar uma nova rodada de treinamento. Durante o treinamento, todas as flores visitadas foram imediatamente substituídas por flores novas, nunca visitadas por outras abelhas, para evitar qualquer sinal químico causado por visitas anteriores das abelhas (Eltz, 2006; Saleh et al., 2007). Após a quinta rodada de treinamento, substituímos todas as 20 flores por cinco flores de cada um dos quatro tipos, [i], [ii], [iii] e [iv], para realizar o experimento. As cinco rodadas de treinamento e o experimento foram realizados sempre no mesmo dia. No caso de testes com abelhas ingênuas, permitimos que as abelhas realizassem apenas um turno de visitas às flores artificiais. Todos os experimentos foram realizados em uma arena de 4x2x2 m (Figura 3A). As flores artificiais foram colocadas aleatoriamente na lateral da arena a cada rodada do experimento (Figura 3B). Contamos as visitas feitas por cada uma das abelhas a cada tipo de flor (Figura 3C). Finalmente, sacrificamos as abelhas após esta rodada de forrageamento.

Para determinar se as abelhas visitaram algum dos tipos de flores com mais frequência, utilizou-se o modelo linear generalizado misto (GLMM) com distribuição de erros de Poisson e estimador de Quadratura de Gauss-Hermite, considerando o tratamento (abelhas ingênuas, abelhas treinadas com flores [i] e abelhas treinadas com flores [ii]) e o tipo de flor como variável fixa e abelha como variável aleatória. Adicionalmente, para verificar se as abelhas visitaram preferencialmente algum dos tipos de flores, em termos de porcentagem de visitas, utilizou-se modelo linear generalizado misto (GLMM) com

distribuição de erro binomial e estimador de Quadratura de Gauss-Hermite, considerando tratamento e tipo de flor como variáveis fixas e abelha como variável aleatória. Além disso, para verificar se os tipos de flores apresentavam a mesma probabilidade de receber a primeira visita das abelhas nas rodadas experimentais, utilizou-se modelo linear generalizado misto (GLMM) com distribuição de erro binomial e estimador de Quadratura de Gauss-Hermite, considerando tratamento e tipo de flor como variável fixa e abelha como variável aleatória.

Figura 3. Desenho experimental para testar o efeito da mudança na simetria e área floral, simulando a florivoria, no comportamento de abelhas polinizadoras do gênero *Bombus*. A. Arena onde os experimentos foram realizados, na 'Estación Experimental de Zonas Áridas', Almeria, Espanha. B. Exemplo de arranjo aleatório de flores dentro da arena. C. Exemplo de visita da abelha *Bombus terrestris* a uma flor artificial do tipo [i].

Além disso, determinar se as abelhas visitavam flores simétricas ou assimétricas com maior frequência, verificar se as abelhas visitavam preferencialmente flores simétricas ou assimétricas, em termos de porcentagem de visitas, e verificar se flores simétricas e assimétricas apresentavam a mesma probabilidade de receber a primeira visita das abelhas nas rodadas experimentais, utilizamos os mesmos três modelos citados com suas respectivas distribuições de erro descritas, considerando tratamento e tipo de simetria floral como variáveis fixas e abelha como variável aleatória.

Por fim, determinar se as abelhas visitavam com maior frequência flores com áreas padrão ou reduzidas, verificar se as abelhas visitavam preferencialmente flores com áreas padrão ou reduzidas, em termos de porcentagem de visitas, e verificar se flores com áreas de superfície padrão e reduzidas apresentavam a mesma probabilidade de receber a primeira visita das abelhas nas rodadas experimentais, utilizamos os mesmos três modelos citados com suas respectivas distribuições de erro descritas, considerando tratamento e área de superfície floral como variáveis fixas e abelha como variável aleatória.

Realizamos essa análise no espaço computacional R (R Core Team, 2019) com os pacotes adicionais: car (Fox e Weisberg, 2019), emmeans (Lenth, 2020), fitdistrplus (Delignette-Muller e Dutang, 2015), ggplot2 (Wickham, 2016), glmmADMB (Fournier et al., 2012; Skaug et al., 2016), lattice (Sarkar, 2008), lme4 (Bates et al., 2015), MASS (Venables e Ripley, 2002) e R2admb (Bolker et al., 2020).

RESULTADOS

Foram computadas as visitas de 42 abelhas, divididas entre os três tratamentos ($\mathbf{N}_{\textit{naïve}}$ = 15, $N_{\text{treinadas com flores [i]}}$ = 14, $N_{\text{treinadas com flores [ii]}}$ = 13). Descobrimos que aproximadamente 95% das abelhas visitaram de três a quatro tipos de flores artificiais apresentadas a elas, independentemente d e sua experiência anterior. Observamos que os diferentes tipos de flores receberam quantidades semelhantes de visitas das abelhas em termos de número de visitas e porcentagem relativa de visitas, independentemente da experiência anterior das abelhas, ou seja, do tratamento a que foram submetidas ($p > 0,05$; ver Anexos 1 e 2 para detalhes estatísticos). Além disso, notamos que a probabilidade de um determinado tipo de flor ser o primeiro a ser visitado por uma abelha também é independente de sua experiência anterior ($p > 0,05$; veja o Apêndice 3 para detalhes estatísticos). Além disso, observamos que a perda da zigomorfia floral não afetou o comportamento das abelhas em relação ao número de visitas realizadas a cada um dos tipos florais, bem como em termos de porcentagem de visitas e na escolha do primeiro tipo floral a ser visitado, independentemente da experiência anterior das abelhas ($p > 0,05$; ver Anexos 4 a 6 para detalhes estatísticos). Além disso, observamos que a diminuição da área floral não afetou o comportamento das abelhas em termos de número de visitas feitas a cada um dos tipos florais, nem em termos de porcentagem de visitas a cada tipo floral ($p > 0,05$; ver Anexos 7 e 8 para detalhes estatísticos). No entanto, abelhas treinadas com flores modelo [ii] fizeram suas primeiras visitas a flores cujas áreas não foram reduzidas

pela florivoria, independentemente de essas flores terem ou não sua zigomorfia intacta ($p < 0,05$; Figura 4; ver Apêndice 9 para estatísticas detalhadas).

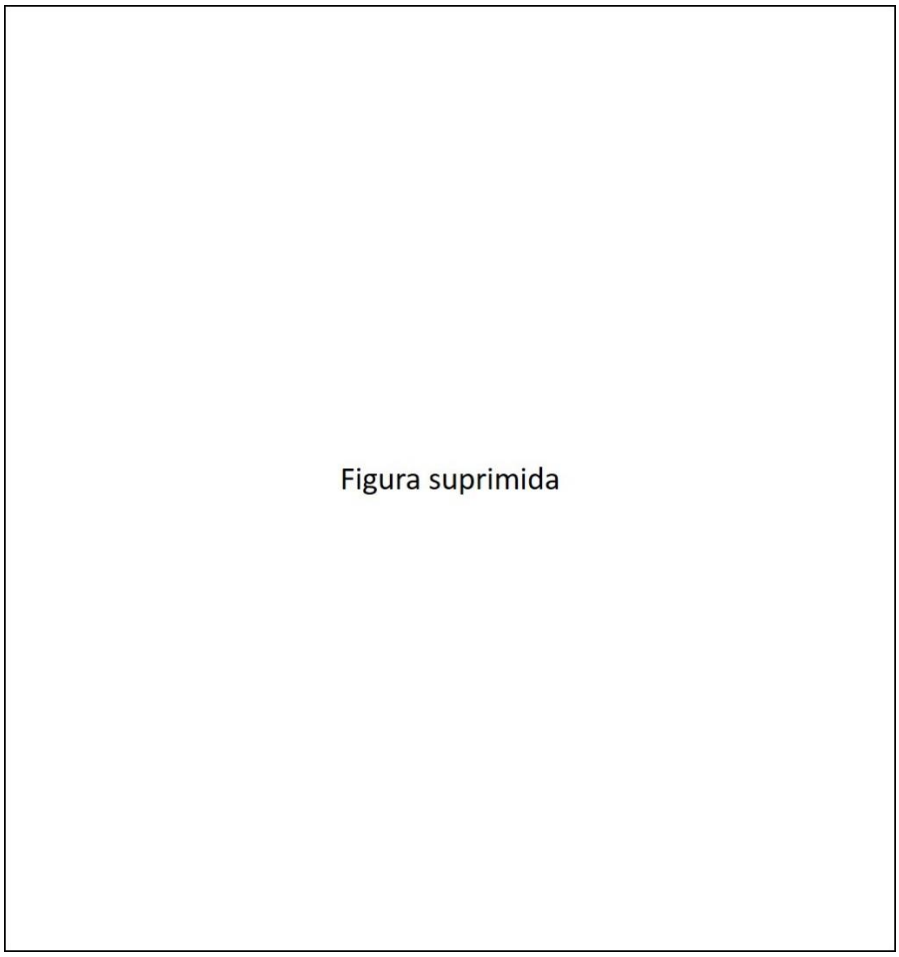

Figura 4. Probabilidade de abelhas submetidas aos três tratamentos (abelhas *naïves*, abelhas treinadas com flores [i] e abelhas treinadas com flores [ii]) fazerem sua primeira visita a flores com área padrão (flores [i] e [iv]) ou com área reduzida (flores [ii] e [iii]). Observe que as abelhas treinadas com flores do tipo [ii] fizeram suas primeiras visitas predominantemente às flores cujas áreas não foram reduzidas pela florivoria, enquanto as abelhas submetidas aos outros dois tratamentos não mostraram preferência (ver Anexo 9 para detalhes estatísticos). As linhas representam a curva suavizada da probabilidade das abelhas submetidas aos três tratamentos fazerem sua primeira visita a flores com área padrão ou com área reduzida pelo método LOESS (regressão ponderada localmente) com intervalo de confiança de 95%.

DISCUSSÃO

Em nosso delineamento experimental, fomos capazes de isolar dois componentes dos sinais visuais florais comumente modificados por florivoria, simetria floral e área da

superfície floral. Além disso, fomos capazes de explorar se, e como, o comportamento de visita das abelhas muda em resposta a mudanças promovidas pela florivoria. Os três tratamentos aos quais submetemos as abelhas em nosso estudo permitiram uma compreensão abrangente das preferências inatas das abelhas e do aprendizado condicionado em relação à simetria e à área floral. Mostramos que a maioria dos indivíduos de abelhas (95%) visitou de três a quatro tipos de flores artificiais, independentemente de sua experiência anterior. Além disso, mostramos que o comportamento das abelhas não foi afetado pelas mudanças na simetria floral simulando as promovidas pela florivoria, independentemente da experiência anterior das abelhas. Por outro lado, as abelhas que forrageiam em flores com área floral reduzida apresentaram maior probabilidade de realizar sua primeira visita a uma flor com área floral maior, independentemente do tipo de simetria apresentada pelas flores. Já as abelhas *naïve* e as abelhas que forrageavam em flores com área floral padrão realizaram suas primeiras visitas às flores com área floral padrão e reduzida, indistintamente.

As combinações de caracteres florais podem atuar como uma única informação integrada, o que pode levar a mudanças de comportamento das abelhas apenas quando integrados, principalmente quando a combinação de caracteres leva a maiores custos energéticos de forrageamento para as abelhas (Gegear et al., 2017). No entanto, se a combinação de características é crucial para a tomada de decisão das abelhas, as mudanças comportamentais mencionadas não são observadas quando cada característica floral é considerada isoladamente (Gegear et al., 2017). Diante disso, pode-se esperar que as mudanças nas pistas visuais florais promovidas pela florivoria, atuem em consonância com a percepção dos polinizadores. No entanto, quando consideramos as duas características concomitantemente (interação entre simetria floral e área de superfície floral, tratada como "tipos de flores"), observamos que a visitação de abelhas não é afetada de forma alguma pela combinação de mudanças na simetria floral e área de superfície floral, independentemente do tratamento ao qual as abelhas foram submetidas.

Nosso estudo também nos permitiu investigar as preferências inatas das abelhas em relação à simetria e ao tamanho floral, observando as respostas de abelhas *naïve*. No entanto, as preferências inatas podem ser suprimidas pelo aprendizado associativo ao longo da vida da abelha (Gumbert, 2000). Assim, as abelhas podem aprender a associar uma determinada sugestão floral com a presença de recurso floral, o que lhes permite fazer uma previsão e testá-la visitando flores que apresentam uma determinada pista (Giurfa, 2007). Este último cenário é mais semelhante ao ambiente natural, no qual os

sinais florais podem ser afetados por fatores abióticos e bióticos, assim como por outros parceiros interagentes, como seria o caso da florivoria em sistemas naturais.

As rodadas experimentais com abelhas *naïve* revelaram que essas abelhas não apresentaram preferência inata em relação às flores zigomorfas, semelhante ao observado por Giurfa et al. (1996) e por West e Laverty (1998) e ao contrário do observado por Rodriguez et al. (2004) em relação aos padrões zigomorfos em flores. Nossa descoberta indica que, em um cenário natural, em que ocorre florivoria, as abelhas *naïve* não negligenciariam flores danificadas devido à perda de simetria zigomorfa. Além disso, as abelhas treinadas com ambos os tipos de flores [i] e [ii] não mostraram preferência por flores simétricas, semelhante ao observado por West e Laverty (1998). Por outro lado, alguns estudos mostraram que as abelhas preferem visitar flores simétricas (Moller, 1995; Moller e Eriksson, 1995; Goulson et al., 2007). No entanto, nesses sistemas, a simetria floral é uma proxy precisa do recurso floral (Moller, 1995; Moller e Eriksson, 1995; Goulson et al., 2007). Portanto, essa preferência por flores simétricas pode ser um comportamento aprendido que é reforçado pelo recurso floral, como no condicionamento clássico (Pavlov, 1927). Além disso, Giurfa et al. (1996) mostraram que as abelhas aprenderam a associar recompensas mais rapidamente com formas simétricas do que com formas assimétricas, indicando uma predisposição para aprender imagens simétricas. Porém, em nossa montagem experimental, sempre oferecemos a mesma quantidade e qualidade de solução açucarada em cada flor, independentemente de sua simetria floral, o que não permite que esse aprendizado aconteça. Embora a zigomorfia esteja associada a sistemas especializados de polinização, sabe-se que espécies de plantas com esta característica floral apresentam menor variação fenotípica (Armbruster et al., 1999; Wolfe e Krstolic, 1999; Ushimaru et al., 2006; Meng et al., 2008) e nossos resultados sugerem que a perda de simetria devido à florivoria não seria prejudicial à atração de polinizadores. Ao considerar a importância da área floral para a escolha das flores pelas abelhas, foi demonstrado que o tempo de busca floral aumenta quando o tamanho floral diminui (Spaethe et al., 2001), o que é esperado uma vez que as abelhas ajustam sua velocidade de voo em cenários visualmente desafiadores para reduzir o risco de perda de flores (Chittka e Spaethe, 2007). Portanto, a relevância desse traço floral no aumento do tempo de busca floral é dependente da paisagem, sendo mais forte quando as flores estão imersas em um fundo homogêneo (Telles et al., 2017). Vários estudos mostram que as abelhas realizam mais visitas a flores maiores (Galen e Newport, 1987; Galen, 1989; Cresswell e Galen, 1991; Eckhart, 1991; Cresswell e Galen, 1991; Ohara e Higashi, 1994). Por outro

lado, Cresswell e Robertson (1994) não encontraram relação entre tamanho floral e visitação floral em abelhas experientes. No entanto, nenhum desses estudos foi realizado com abelhas *naïve*. Até onde sabemos, este é o primeiro estudo a mostrar que abelhas *naïve* não mostraram preferência por flores com maior área de superfície. Nossos experimentos com abelhas treinadas mostraram resultados contrastantes. Enquanto as abelhas treinadas com flores de tamanho padrão não mostraram preferência por flores de tamanho padrão sobre flores com área reduzida, as abelhas treinadas com flores de área reduzida preferencialmente realizaram sua primeira visita a flores de tamanho padrão, que eram maiores que aquelas com as quais as abelhas foram treinadas. Este resultado indica que as abelhas de fato preferem visitar flores maiores, como já havia sido encontrado para abelhas com experiência de forrageamento livre (Cresswell e Galen, 1991; Eckhart, 1991; Ohara e Higashi, 1994), o que pode ser devido ao fato que muitos polinizadores preferem visitar flores maiores (Harper, 1977; Stephenson, 1979; Willson et al., 1979; Wyatt, 1980; Shannon e Wyatt, 1986). No entanto, Goulson (2010) levantou a hipótese de que as maiores taxas de visitação encontradas em estudos com abelhas com experiência de forrageamento livre podem ser devido ao fato de que o tamanho da flor pode estar correlacionado com a produção de pólen ou néctar, de forma que a seleção de grandes flores pelas abelhas seria reforçada pelo aprendizado (Cresswell e Galen, 1991; Duffield et al., 1993; Gomez et al., 2008), pois flores grandes produziriam mais recursos florais. Por outro lado, em nosso estudo, todas as flores produziram a mesma qualidade e quantidade de néctar independente de sua área. Assim, pode não ter havido nenhuma aprendizagem associada ao recurso pelas abelhas, o que pode explicar por que registramos maior probabilidade de flores maiores receberem a primeira visita, mas isso não implicou em maior número de visitas a flores maiores.

Portanto, os efeitos da florivoria no comportamento das abelhas são dependentes do contexto. Em cenários naturais onde a florivoria é rara, assim como os resultados encontrados para abelhas treinadas com flores intactas, as abelhas visitariam flores danificadas e intactas independentemente de sua simetria ou área de superfície. Em cenários naturais onde a florivoria é comum, em que as abelhas estariam acostumadas a forragear em flores danificadas com corolas reduzidas, elas podem preferir visitar flores intactas depois de visitar várias flores danificadas, mas isso não se traduzirá em negligenciar flores danificadas, especialmente se os recursos florais não são afetados pela florivoria. Consequentemente, embora a florivoria afete as características visuais florais, como a simetria floral e a área da corola, as alterações nelas causadas (individual ou

concomitantemente) não afetam a comunicação planta-polinizador e, portanto, não desencorajam a visitação das abelhas.

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Apêndice 1. Contrasts of the generalized linear mixed model (number of visits ~ treatment * flower type + (1|bee)) used to test whether bees preferentially visited any of the artificial flower types in terms of number of visits performed by bees submitted to three different training methods (treatments) to the four types of artificial flowers.

Flower type	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
[i]	-	Naive-Trained with flower [i]	0.1942	0.250	0.775	0.7183
	-	Naive-Trained with flower [ii]	0.3528	0.246	1.436	0.3223
	-	Trained with flower [i]-[ii]	0.1587	0.238	0.668	0.7822
[ii]	-	Naive-Trained with flower [i]	0.0134	0.236	0.057	0.9982
	-	Naive-Trained with flower [ii]	-0.1005	0.248	-0.405	0.9134
	-	Trained with flower [i]-[ii]	-0.1139	0.251	-0.454	0.8927
[iii]	-	Naive-Trained with flower [i]	0.2101	0.238	0.883	0.6511
	-	Naive-Trained with flower [ii]	-0.0576	0.259	-0.222	0.9732
	-	Trained with flower [i]-[ii]	-0.2676	0.252	-1.063	0.5369
[iv]	-	Naive-Trained with flower [i]	0.1018	0.256	0.397	0.9166
	-	Naive-Trained with flower [ii]	0.1431	0.258	0.554	0.8443
	-	Trained with flower [i]-[ii]	0.0413	0.256	0.161	0.9858
-	Naive	Flower type [i]-[ii]	-0.2097	0.246	-0.854	0.8286
-		Flower type [i]-[iii]	-0.0953	0.252	-0.378	0.9816
-		Flower type [i]-[iv]	0.0000	0.258	0.000	1.0000
-		Flower type [ii]-[iii]	0.1144	0.239	0.478	0.9640
-		Flower type [ii]-[iv]	0.2097	0.246	0.854	0.8286
-		Flower type [iii]-[iv]	0.0953	0.252	0.378	0.9816
-	Trained with flower [i]	Flower type [i]-[ii]	-0.0290	0.241	-0.120	0.9994
-		Flower type [i]-[iii]	-0.1112	0.236	-0.471	0.9654
-		Flower type [i]-[iv]	0.0924	0.248	0.372	0.9824
-		Flower type [ii]-[iii]	-0.0822	0.234	-0.351	0.9852
-		Flower type [ii]-[iv]	0.1214	0.247	0.492	0.9609
-		Flower type [iii]-[iv]	0.2036	0.242	0.841	0.8348
-	Trained with flower [ii]	Flower type [i]-[ii]	0.2436	0.248	0.982	0.7596
-		Flower type [i]-[iii]	0.3151	0.253	1.245	0.5982
-		Flower type [i]-[iv]	0.2097	0.246	0.854	0.8286
-		Flower type [ii]-[iii]	0.0715	0.267	0.267	0.9933
-		Flower type [ii]-[iv]	-0.0339	0.260	-0.130	0.9992
-		Flower type [iii]-[iv]	-0.1054	0.265	-0.397	0.9788

Apêndice 2. Contrasts of the generalized linear mixed model (percentage of visits ~ treatment * flower type + (1|bee)) used to test whether bees preferentially visited any of the artificial flower types in terms of percentage of visits performed by bees submitted to three different training methods (treatments) to the four types of artificial flowers.

Flower type	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
[i]	-	Naive-Trained with flower [i]	0.2390	0.865	0.276	0.9588
	-	Naive-Trained with flower [ii]	0.6861	0.846	0.811	0.6964
	-	Trained with flower [i]-[ii]	0.4471	0.835	0.536	0.8537
[ii]	-	Naive-Trained with flower [i]	-0.3122	0.845	-0.370	0.9274
	-	Naive-Trained with flower [ii]	-0.5736	0.901	-0.637	0.8000
	-	Trained with flower [i]-[ii]	-0.2614	0.942	-0.277	0.9585
[iii]	-	Naive-Trained with flower [i]	0.3059	0.851	0.360	0.9312
	-	Naive-Trained with flower [ii]	-0.1205	0.916	-0.132	0.9905
	-	Trained with flower [i]-[ii]	-0.4265	0.901	-0.473	0.8838
[iv]	-	Naive-Trained with flower [i]	-0.2392	0.892	-0.268	0.9611
	-	Naive-Trained with flower [ii]	-0.0798	0.888	-0.090	0.9956
	-	Trained with flower [i]-[ii]	0.1594	0.931	0.171	0.9840
-	Naive	Flower type [i]-[ii]	-0.3889	0.838	-0.464	0.9669
-		Flower type [i]-[iii]	-0.0389	0.872	-0.045	1.0000
-		Flower type [i]-[iv]	-0.1332	0.861	-0.155	0.9987
-		Flower type [ii]-[iii]	0.3500	0.833	0.420	0.9751
-		Flower type [ii]-[iv]	0.2557	0.822	0.311	0.9896
-		Flower type [iii]-[iv]	-0.0943	0.857	-0.110	0.9995
-	Trained with flower [i]	Flower type [i]-[ii]	0.1623	0.871	0.186	0.9977
-		Flower type [i]-[iii]	-0.1058	0.844	-0.125	0.9993
-		Flower type [i]-[iv]	0.3450	0.895	0.385	0.9805
-		Flower type [ii]-[iii]	-0.2682	0.862	-0.311	0.9896
-		Flower type [ii]-[iv]	0.1827	0.912	0.200	0.9972
-		Flower type [iii]-[iv]	0.4509	0.886	0.509	0.9570
-	Trained with flower [ii]	Flower type [i]-[ii]	0.8708	0.909	0.958	0.7731
-		Flower type [i]-[iii]	0.7677	0.892	0.860	0.8253
-		Flower type [i]-[iv]	0.6327	0.873	0.725	0.8873
-		Flower type [ii]-[iii]	-0.1031	0.978	-0.105	0.9996
-		Flower type [ii]-[iv]	-0.2381	0.961	-0.248	0.9947
-		Flower type [iii]-[iv]	-0.1350	0.946	-0.143	0.9990

Apêndice 3. Contrasts of the generalized linear mixed model (probability of first visit ~ treatment * flower type + (1|bee)) used to test whether the bees submitted to the three different training methods (treatments) performed the first visit preferentially to one of the artificial flower types.

Flower type	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
[i]	-	Naive-Trained with flower [i]	-0.288	0.875	-0.329	0.9421
	-	Naive-Trained with flower [ii]	0.857	0.806	1.063	0.5370
	-	Trained with flower [i]-[ii]	1.145	0.857	1.337	0.3747
[ii]	-	Naive-Trained with flower [i]	-1.386	0.928	-1.494	0.2938
	-	Naive-Trained with flower [ii]	-2.079	1.167	-1.782	0.1755
	-	Trained with flower [i]-[ii]	-0.693	1.291	-0.537	0.8531
[iii]	-	Naive-Trained with flower [i]	1.386	0.838	1.654	0.2231
	-	Naive-Trained with flower [ii]	-0.318	1.004	-0.317	0.9460
	-	Trained with flower [i]-[ii]	-1.705	0.936	-1.821	0.1628
[iv]	-	Naive-Trained with flower [i]	0.080	1.077	0.074	0.9970
	-	Naive-Trained with flower [ii]	1.061	0.969	1.095	0.5169
	-	Trained with flower [i]-[ii]	0.981	0.972	1.009	0.5710
-	Naive	Flower type [i]-[ii]	-0.606	0.787	-0.771	0.8677
-		Flower type [i]-[iii]	0.375	0.870	0.430	0.9733
-		Flower type [i]-[iv]	0.860	0.958	0.898	0.8059
-		Flower type [ii]-[iii]	0.981	0.833	1.177	0.6414
-		Flower type [ii]-[iv]	1.466	0.924	1.586	0.3865
-		Flower type [iii]-[iv]	0.486	0.997	0.487	0.9620
-	Trained with flower [i]	Flower type [i]-[ii]	0.492	1.004	0.491	0.9612
-		Flower type [i]-[iii]	-1.299	0.843	-1.542	0.4123
-		Flower type [i]-[iv]	0.492	1.004	0.491	0.9612
-		Flower type [ii]-[iii]	-1.792	0.932	-1.922	0.2187
-		Flower type [ii]-[iv]	0.000	1	0.000	1.0000
-		Flower type [iii]-[iv]	1.792	0.932	1.922	0.2187
-	Trained with flower [ii]	Flower type [i]-[ii]	2.331	1.180	1.975	0.1975
-		Flower type [i]-[iii]	1.551	0.949	1.634	0.3593
-		Flower type [i]-[iv]	0.657	0.819	0.802	0.8535
-		Flower type [ii]-[iii]	-0.780	1.294	-0.603	0.9312
-		Flower type [ii]-[iv]	-1.674	1.202	-1.393	0.5038
-		Flower type [iii]-[iv]	-0.894	0.976	-0.916	0.7963

Apêndice 4. Contrasts of the generalized linear mixed model (number of visits ~ treatment * symmetry type + (1|bee)) used to test whether bees preferentially visited symmetric or asymmetric flowers in terms of number of visits performed by bees submitted to three different training methods (treatments).

Symmetry type	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
Asymmetric	-	Naive-Trained with flower [i]	0.0540	0.173	0.311	0.9480
	-	Naive-Trained with flower [ii]	0.0159	0.179	0.089	0.9956
	-	Trained with flower [i]-[ii]	-0.0380	0.179	-0.212	0.9755
Zygomorphic	-	Naive-Trained with flower [i]	0.2025	0.173	1.174	0.4688
	-	Naive-Trained with flower [ii]	0.1588	0.177	0.895	0.6434
	-	Trained with flower [i]-[ii]	-0.0437	0.172	-0.254	0.9650
-	Naive	Zygomorphic-Asymmetric	0.0616	0.175	0.351	0.7258
-	Trained with flower [i]	Zygomorphic-Asymmetric	-0.0870	0.170	-0.511	0.6096
-	Trained with flower [ii]	Zygomorphic-Asymmetric	-0.0813	0.180	-0.451	0.6522

Apêndice 5. Contrasts of the generalized linear mixed model (percentage of visits ~ treatment * symmetry type + (1|bee)) used to test whether bees preferentially visited symmetric and asymmetric flowers in terms of percentage of visits performed by bees submitted to three different training methods (treatments).

Symmetry type	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
Asymmetric	-	Naive-Trained with flower [i]	-0.2771	0.612	-0.452	0.8933
	-	Naive-Trained with flower [ii]	-0.3263	0.630	-0.518	0.8627
	-	Trained with flower [i]-[ii]	-0.0492	0.661	-0.075	0.9969
Zygomorphic	-	Naive-Trained with flower [i]	0.2730	0.606	0.450	0.8943
	-	Naive-Trained with flower [ii]	0.3143	0.615	0.511	0.8659
	-	Trained with flower [i]-[ii]	0.0413	0.605	0.068	0.9974
-	Naive	Zygomorphic-Asymmetric	0.245	0.598	0.410	0.6819
-	Trained with flower [i]	Zygomorphic-Asymmetric	-0.305	0.620	-0.491	0.6231
-	Trained with flower [ii]	Zygomorphic-Asymmetric	-0.395	0.646	-0.612	0.5405

Apêndice 6. Contrasts of the generalized linear mixed model (probability of first visit ~ treatment * symmetry type + (1|bee)) used to test whether the bees submitted to the three different training methods (treatments) performed the first visit preferentially to symmetric or asymmetric flowers.

Symmetry type	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
Asymmetric	-	Naive-Trained with flower [i]	-0.780	0.680	-1.148	0.4847
	-	Naive-Trained with flower [ii]	-0.423	0.647	-0.655	0.7896
	-	Trained with flower [i]-[ii]	0.357	0.734	0.486	0.8781
Zygomorphic	-	Naive-Trained with flower [i]	0.602	0.585	1.029	0.5584
	-	Naive-Trained with flower [ii]	0.379	0.606	0.625	0.8063
	-	Trained with flower [i]-[ii]	-0.223	0.580	-0.385	0.9216
-	Naive	Zygomorphic-Asymmetric	0.178	0.597	0.298	0.7657
-	Trained with flower [i]	Zygomorphic-Asymmetric	-1.204	0.669	-1.800	0.0718
-	Trained with flower [ii]	Zygomorphic-Asymmetric	-0.624	0.654	-0.954	0.3402

Apêndice 7. Contrasts of the generalized linear mixed model (number of visits ~ treatment * floral area + (1|bee)) used to test whether bees preferentially visited symmetric or asymmetric flowers in terms of number of visits performed by bees submitted to three different training methods (treatments).

Area	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
Standard	-	Naive-Trained with flower [ii]	0.253	0.178	1.426	0.3275
	-	Naive-Trained with flower [i]	0.149	0.179	0.832	0.6828
	-	Trained with flower [i]-[ii]	0.104	0.174	0.600	0.8202
Reduced	-	Naive-Trained with flower [i]	0.111	0.167	0.663	0.7848
	-	Naive-Trained with flower [ii]	-0.080	0.179	-0.446	0.8960
	-	Trained with flower [i]-[ii]	-0.191	0.178	-1.075	0.5295
-	Naive	Reduced area-Standard area	-0.154	0.176	-0.876	0.3809
-	Trained with flower [i]	Reduced area-Standard area	-0.116	0.171	-0.681	0.4961
-	Trained with flower [ii]	Reduced area-Standard area	0.179	0.181	0.991	0.3219

Apêndice 8. Contrasts of the generalized linear mixed model (percentage of visits ~ treatment * floral area + (1|bee)) used to test whether bees preferentially visited symmetric and asymmetric flowers in terms of percentage of visits performed by bees submitted to three different training methods (treatments).

Floral area	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
Standard	-	Naive-Trained with flower [i]	0.00657	0.619	0.011	0.9999
	-	Naive-Trained with flower [ii]	0.32226	0.607	0.531	0.8563
	-	Trained with flower [i]-[ii]	0.31569	0.617	0.511	0.8659
Reduced	-	Naive-Trained with flower [i]	-0.00610	0.596	-0.010	0.9999
	-	Naive-Trained with flower [ii]	-0.35347	0.641	-0.552	0.8456
	-	Trained with flower [i]-[ii]	-0.34737	0.651	-0.534	0.8546
-	Naive	Reduced area-Standard area	-0.153	0.597	-0.257	0.7973
-	Trained with flower [i]	Reduced area-Standard area	-0.141	0.618	-0.228	0.8199
-	Trained with flower [ii]	Reduced area-Standard area	0.522	0.650	0.803	0.4217

Apêndice 9. Contrasts of the generalized linear mixed model (probability of first visit ~ treatment * floral area + (1|bee)) used to test whether the bees submitted to the three different training methods (treatments) performed the first visit preferentially to symmetric or asymmetric flowers. We considered the p-values in bold in the table as statistically significant (α = 95%).

Floral area	Treatment	Contrast	Estimate	SE	Z-ratio	P-value
Standard	-	Naive-Trained with flower [i]	-0.140	0.672	-0.208	0.9764
	-	Naive-Trained with flower [ii]	0.916	0.609	1.505	0.2887
	-	Trained with flower [i]-[ii]	1.056	0.637	1.657	0.2218
Reduced	-	Naive-Trained with flower [i]	0.100	0.568	0.176	0.9830
	-	Naive-Trained with flower [ii]	-1.190	0.732	-1.626	0.2348
	-	Trained with flower [i]-[ii]	-1.290	0.735	-1.754	0.1853
-	Naive	Reduced area-Standard area	-0.539	0.606	-0.890	0.3737
-	Trained with flower [i]	Reduced area-Standard area	-0.779	0.638	-1.220	0.2223
-	Trained with flower [ii]	Reduced area-Standard area	1.567	0.734	2.134	**0.0329**

CAPÍTULO 3

A REDUÇÃO DA ÁREA DE OSMÓFOROS POR FLORÍVOROS AFETA A QUANTIDADE TOTAL DE ODOR EMITIDO PELAS FLORES?

A REDUÇÃO DA ÁREA DE OSMÓFOROS POR FLORÍVOROS AFETA A QUANTIDADE TOTAL DE ODOR EMITIDO PELAS FLORES?

ABSTRACT

Volatile compounds emitted from flowers are produced in osmophores, which are responsible for the synthesis and emission of floral scent, an important signal in plant-pollinator communication pathways. Besides attracting pollinators, floral scent can also attract florivores and both groups, acting simultaneously, can have contrasting ecological roles. Florivores, may directly reduce the physical surface of the flower by consuming ormophore-bearing flower portions that are responsible for scent emission. Thus, florivores may negatively impact scent emission by removing sites of volatile synthesis and emission. We identified the areas of the corolla that correspond to osmophores and detailed their structure in plant species with different pollination systems, which have their flower tissues consumed by variable florivore groups. Then, we identified if florivores consumed floral portions bearing osmophores, which might directly affect the amount of scent emission due to secretory tissue removal. To check this assumption, we experimentally tested if the removal of osmophore-containing corolla portions by florivores affected scent emission in these plant species. We found that the sampled species presented osmophores distributed in clustered or diffuse manners, in some species glandular trichome cells were also involved in scent synthesis and emission, and in some species the osmophores were in the uniseriate epidermis, whereas in others, they were in the mesophyll or in the subepidermal position. Furthermore, we verified that florivory only affected the total amount of scent emitted by one species. Our findings suggest that plant species show resilience regarding scent emission, since florivory in variable intensities involving the removal of osmophores-bearing areas did not affect floral scent in most species. The florivory-induced reduction in scent emission in only one of the plant species, together with the absence of response in the other species, may suggest that florivory-induced changes in floral scent are a species-specific response and that most plant species might adjust their metabolism and still emit similar amounts of floral scent despite florivory.

Keywords: chemical signalling, floral scent, florivory, florivory-induced chemical response.

INTRODUCTION

Volatile compounds emitted from flowers are responsible for floral scent that represents an important signal in plant-pollinator communication pathways (Chittka & Thomson, 2001; Dötterl and Jürgens, 2005; Schaefer & Ruxton, 2011). Floral scent is produced in specific secretory structures, osmophores, which may comprise only epidermis or be multi-layered, sometimes with trichomes (Vogel, 1990; Effmert et al. 2006, Hernández & Katinas, 2019). Volatile compounds, most lipophilic, belong to different classes but all of them are characterized by their low molecular weight and low vapour pressure, enough to be released from flower tissues and spread into the air under natural temperature (Knudsen & Gershenzon, 2020).

Besides attracting pollinators, chemical cues can be also tracked by florivores showing similar sensorial abilities to pollinators, and both groups, acting simultaneously, can have contrasting ecological roles (Schaefer & Ruxton, 2011). Florivores, by directly consuming flower portions where osmophores are located, may reduce the physical surface of the flower that is responsible for scent emission and may alter the total amount of scent emitted by flowers (Zangerl & Berenbaum, 2009). The presence of biosynthetic enzymes in specific floral organs and tissues indicates localized volatile synthesis (Dudareva et al., 2003; Effmert et al., 2006). Thus, by consuming floral areas bearing osmophores, florivores may negatively impact scent emission by removing sites of volatile synthesis. Although it is well known that all flower organs are not equally involved in scent emission and that spatial patterns within a flower are common, petals are often the main source of floral scent (Effmert et al. 2006). Additionally, some finds suggest that compounds that are attractive to pollinators may be associated with petal emission (García et al. 2021). In this scenario plant-pollinator communication pathways can be affected, and consequently, pollinators may neglect flowers damaged by florivores due to a reduction in scent emission (Mothershead & Marquis, 2000 and references therein; Malo *et al*., 2001).

In this study, we identified the areas of the corolla that correspond to osmophores and detailed their structure in plant species with different pollination systems, which have their flower tissues consumed by variable florivore groups. Thus, we compared the floral portion bearing osmophores with the natural pattern of florivory, aiming to identify if florivores are consuming these flower portions, which might directly affect the amount of scent emission due to secretory tissue removal. To check this assumption, we

experimentally tested in field conditions if the removal of osmophore-containing corolla portions by florivores indeed affected scent emission in these plant species.

MATERIAL AND METHODS

We investigated scent producing sites using Vogel's neutral red test (Dafni *et al*., 2005) to detect the distribution of osmophores in newly opened flowers (Wiśniewska *et al*., 2018). We sampled three to five fresh flowers/inflorescences per species and maintained every unit in an aqueous solution of neutral red (0.01%, pH 8) for an interval of 30-45 minutes. After this time, we washed every unit in abundant water, analysed them carefully, described the location of the stained areas and photographed the material. Following, we characterized the osmophores through surface micromorphology analyses, anatomy and histolocalization of the main classes of compounds associated to the osmophore secretion. For surface analysis, samples measuring about 1 cm^2, obtained from newly collected flowers, were immediately fixed in Karnovsky's reagent (Karnovsky, 1965) for 24 hours, dehydrated in acetonic series and dried in a critical point equipment (Bal Tec CPD 030). The samples were glued to aluminium sample holders and vacuum metallized with gold (about 10 nm thick) in a Bal-Tec SCD 050 equipment. The analysis and documentation were performed using a scanning electron microscope (SEM) Quanta 200 - FEI (FEI, Hillsboro, OR, USA), at 20 kv, at the Electron Microscopy Center of the Institute of Biosciences of Botucatu, UNESP. For the anatomical characterization of the osmophores and histolocalization of groups of substances associated with floral scent, samples obtained from newly opened flowers were fixed in Karnovsky's reagent (Karnovsky, 1965) for 48 hours, dehydrated in an alcoholic series and embedded in glycol-methacrylate resin (Historesin, Leica Instruments ®). Transverse and longitudinal sections, approximately 5 μm thick, were obtained using a semi-automated rotating microtome (Leica Biosystems RM2245, Wetzlar, Germany), with a disposable steel blade. Sections were stained with 0.05% toluidine blue, pH 4.7 for histological characterization (O'Brien *et al*., 1964), Sudan IV to detect lipidic substances (Johansen, 1940) and NADI reagent to detect terpenoids (David & Carde, 1964). Appropriate controls were run simultaneously for each test. The sections were mounted in acrylic resin and sealed with colourless enamel. Images were obtained with Leica DM5500B photomicroscope with a digital camera attached (Leica DFC 425) and LAS software, at the Laboratory of Plant Anatomy, Institute of Biosciences, UNESP.

We performed around 80 h of field expeditions during daylight and night hours (6:00 to 24:00 h) to characterize the florivory pattern and the most frequent florivores feeding on every plant species. We registered all the florivores observed feeding on flowers and described the patterns of florivory, with focus on the flower portions removed. We classified an animal as the most frequent florivore when we registered its presence, or the damages caused by it, in more than 70% of the events.

We verified if natural florivory caused by the most common florivores of these plant species affected the total amount of floral scent emitted by damaged flowers. For that, we bagged two intact pre-anthesis buds (or inflorescences when flowers were minute) per plant to ensure that pollinators or other florivores could not visit them (the sample number of plants varied according with plant species, see Appendix 1 for details). When the flowers opened, we inserted in one flower per plant a florivore into the bag. We kept the florivores inside the bags, so that they could freely feed on the flowers. After the florivores had eaten, we removed them and sampled the VOCs (volatile organic compounds) emitted by the fed-on flowers. We used the flowers that remained bagged throughout the experiment as positive control. We sampled the scent emitted by these flowers at the same time as we sampled the damaged flowers. Samples were collected following the protocol by Dötterl et al. (2005). The flowers were enclosed in polyethylene bags. The volatile compounds accumulated inside these bags for 10 minutes were collected in small tubes containing an adsorbent mixture, with the aid of an air suction pump with a flow of 200 ml/min. After exhausting the air inside the bags, the circulating air was collected for a variable time adjusted for each species, based on information obtained preliminarily on the specific sampling of each one of them (see number of samples for each plant species in Appendix 1). The adsorbent tubes were made from microtubes with 20 mm of length and 2 mm of internal diameter, filled with a mixture of 1.5 mg Tenax-TA (60 - 80 mesh) and 1.5 mg Carbotrap B (20 - 40 mesh), contained by glass wool. The samples were stored in freezer at approximately -20 °C until the moment of analysis. We analysed the scent samples on an automated thermo desorption system attached to a gas chromatograph coupled to a mass spectrometer (GC-MS). The GC-MS was equipped with a ZB-5 fused silica column (60 m long, 0.25 mm of inner diameter, 0.25 μm of film thickness) and maintained a constant 1.5 mL/min flow of helium as the carrier gas. The injector temperature was 200°C and the samples were injected in variable split modes for each species, based on preliminarily runs of test samples of each one of them. The oven temperature started at 40°C, then increased by 6°C/min to 250°C, at which it kept constant

for 1 min. The MS interface was set at 250°C. Mass spectra were taken at 70 eV (in EI mode), with a scanning range of 30–350 m/z. The data were analysed using the GCMSolution package, Version 4.41 (Shimadzu). We identified volatile compounds by comparing their Kovats retention indices (based on a series of n-alkanes) and mass spectra to data available in the databases ADAMS (Adams, 2007), ESSENTIALOILS-23P, FFNSC 2, Wiley 9 and Nist11. If possible, we confirmed compound identities by comparing their Kovats retention indices to those of authentic reference standards. We excluded from the analyses the compounds that were detected in flowers as well as in leaf controls. To allow the quantitative analysis of floral scent, we injected 100 ng each of ca. 150 components, among them monoterpenes, aliphatic, and aromatic compounds, into the GC/MS system. We used the mean peak area (total ion current) of these compounds to estimate the total amount of floral scent available in each sample, according to Etl *et al*. (2016).

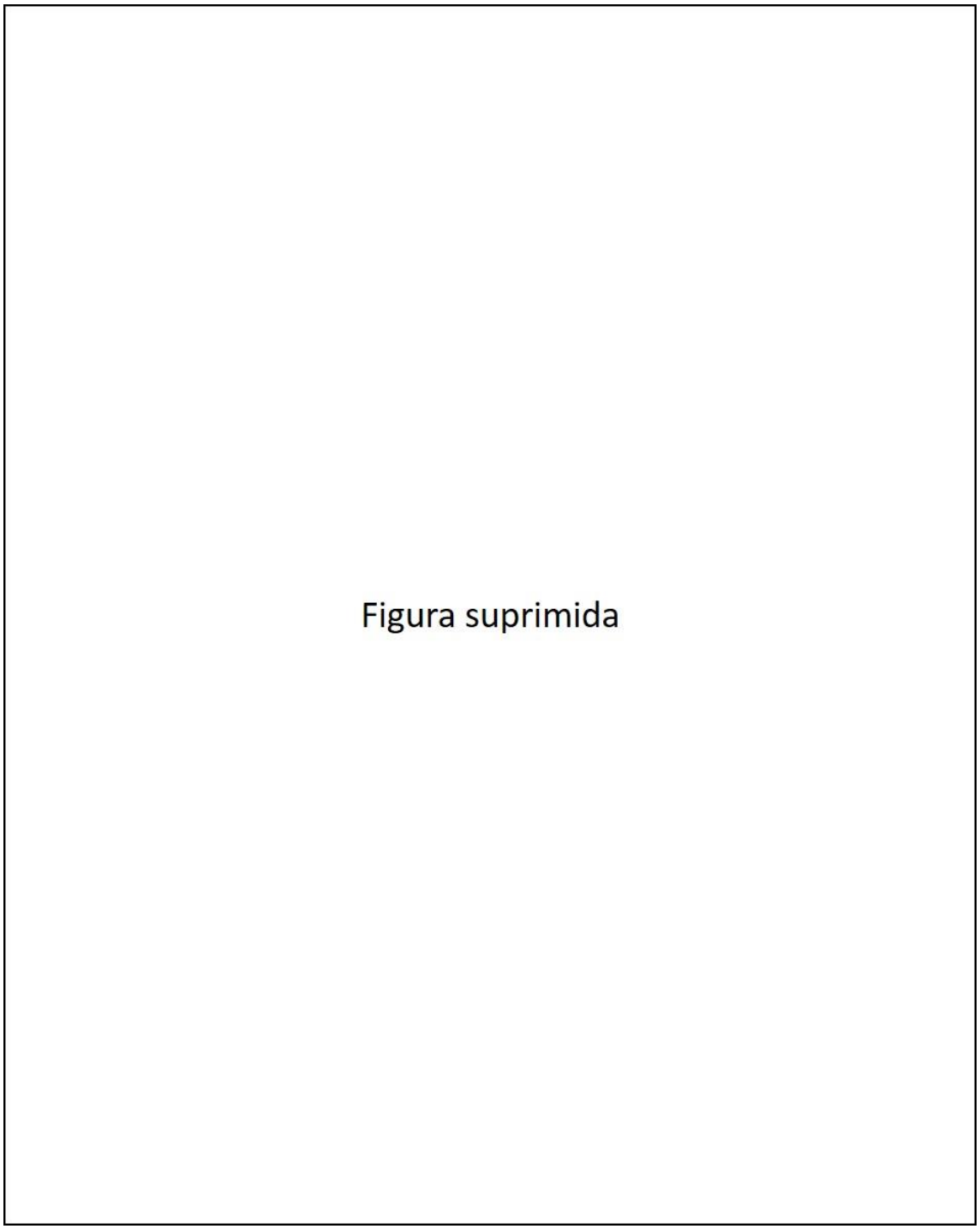
We performed Linear Mixed Models (LMMs) to evaluate if the total amount of scent emitted by flowers that were fed on by florivores differed from that of intact flowers, considering treatment as a fixed factor and plant individual as a random factor. For that, we used R v. 4.0.2 (R Core Team, 2020) with additional packages: lme4 (Bates et al., 2015) and lmerTest (Kuznetsova *et al.*, 2017).

RESULTS

Neutral red tests indicated the presence of metabolically active cells in bracts, and in all the flower whorls, mainly in corolla, which were distributed in clustered or diffuse manners (Figure 1A-H). The anatomical analysis showed that the regions stained with neutral red corresponded to the osmophores (Figure 2A-I). In species bearing trichomes (Figure 2A, C, D, I), the production of terpenes, total lipids and polysaccharides were detected in glandular trichome cells. Other species showed osmophores in the uniseriate epidermis (Figure 2B, E-G) or in the mesophyll, in the subepidermal position (Figure 2H). The epidermal cells involved in the production of scent were invariably more voluminous and had a denser content than the common cells of the epidermis, exhibiting shapes ranging from rectangular (Figure 2F), globose (Figure 2E-G) to papillary (Figure 2H). The cuticle ranged from smooth (Figure 2A) to striated (Figure 2E).

We verified that in seven of the eight sampled species, the florivores feed on flower portions that bear osmophores (Table 1, Figure 1A-C, E-H), directly reducing the area responsible for scent emission. The only exception was *Lantana camara* that had

osmophores placed at the entrance of the corolla tube, showing a ring-like shape, but florivory was registered only on lobe margins and at the basis of the corolla tube (Table 1, Figure 1D), both portions lacking osmophores. Thus, in this species the area responsible for scent emission was not affected by florivory.



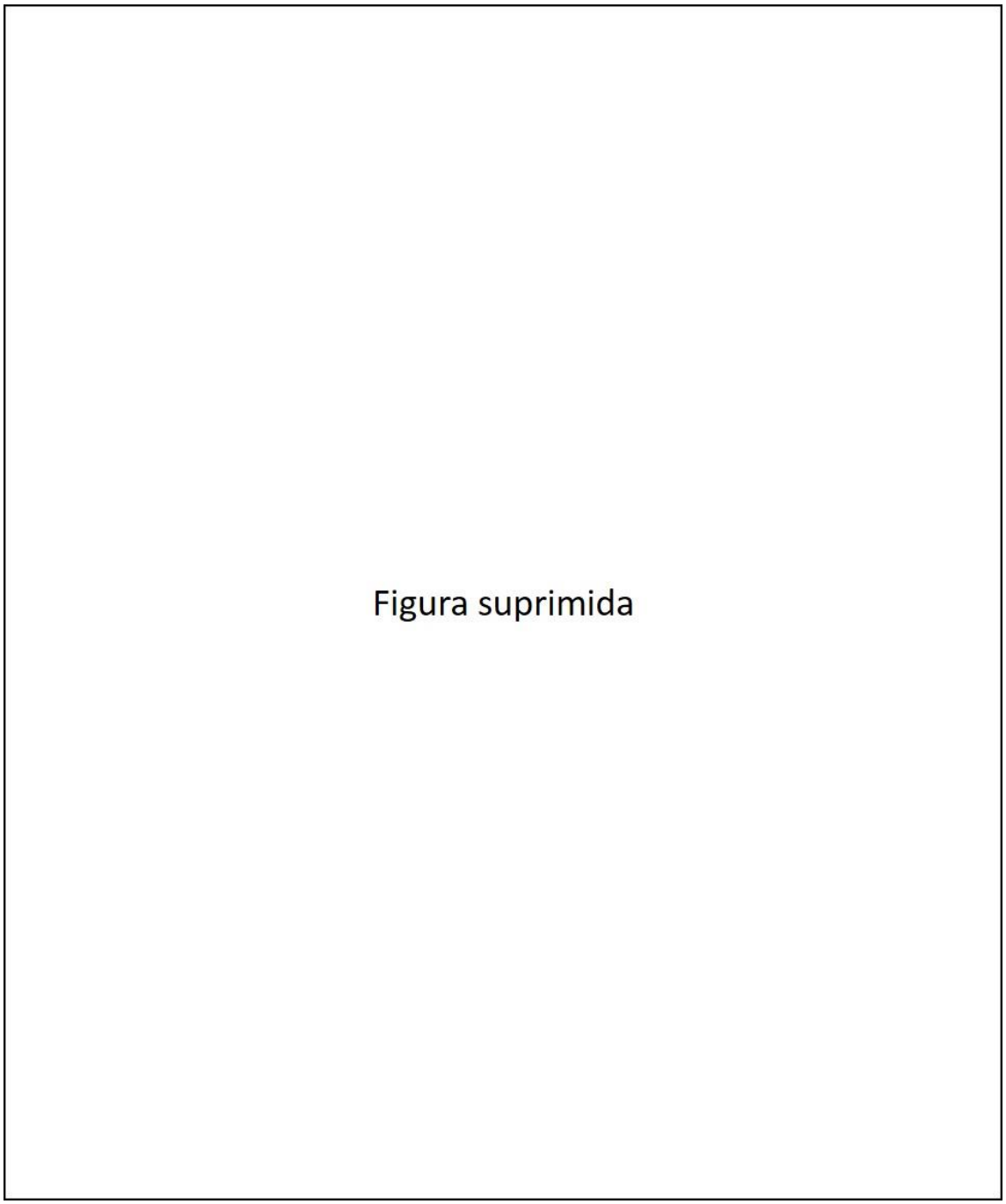


Figure 1. Intact flowers (1st column), flowers stained with Vogel's neutral red solution (2nd column) and flowers that were fed on by florivores, showcasing the most frequent florivory pattern registered in the field (3rd column). Row A: *Amphilophium elongatum*. Row B: *Byrsonima intermedia*. Row C: *Centrosema pubescens*. Row D: *Lantana camara*. Row E: *Lippia alba*. Row F: *Pyrostegia venusta*. Row G: *Tocoyena formosa.* Row H: *Zeyheria montana*.

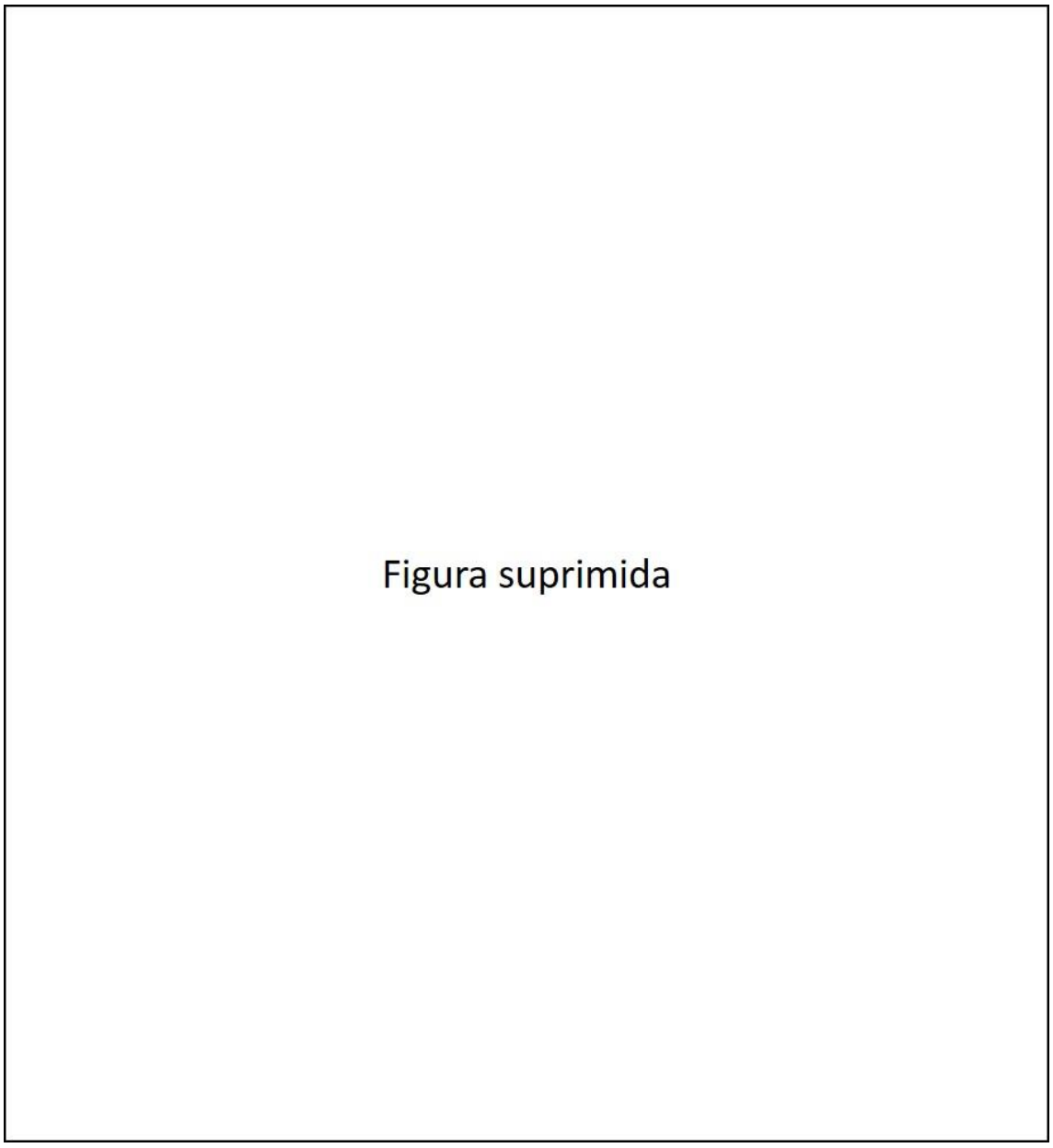


Figure 2. Anatomical characterization of osmophores in tropical plant species. A. *Amphilophium elongatum.* Peltate glandular trichome with secretion externally disposed on head cells (*). B. *Byrsonima intermedia.* Epidermal cells showing positive reaction for terpenes (arrow heads). C. *Centrosema pubescens.* Trichome with positive reaction with NADI reagent. D. *Lantana camara.* Epidermal cell showing positive reaction to NADI (arrow). E-F. *Lippia alba.* Papilous epidermis and trichomes showing positive reaction to NADI, note secretion accumulated on surface of tubular portion. G. *Pyrostegia venusta.* Epidermal cells showing terpenes stained with NADI reagent. H. *Tocoyena formosa.* Terpene accumulated in epidermal and subepidermal cells (arrows) and starch grains (arrowheads). I. *Zeyheria montana.* Peltate glandular trichomes with terpene droplets accumulated in head cells (arrow) revealed by NADI reagent.

Table 1. Plant species from savanna physiognomies of Cerrado, flower portions bearing osmophores, florivores, flower portions commonly fed on by florivores and visually estimated area removed by the most common florivore. The most frequent or the only florivore group observed for each plant species are in bold in the third column.

Plant species	**Osmophores**	**Florivores and feeding pattern**	**Area removed (%)**
Amphilophium mansoanum (DC.) L.G.Lohmann (Bignoniaceae)	Half upper part of corolla tube, above the tube curve and lobes margins	**Beetles** made holes on dorsal and lateral portions of corolla tube, just above the tube curve	**< 5**
Byrsonima intermedia A. Juss. (Malpighiaceae)	Petals and styles	Beetles and **caterpillars** displayed the same pattern eating all the petals and stamens	> 50
Centrosema pubescens Benth. (Leguminosae)	The entire margin of banner and spots spread over all the petals	**Beetles** made small holes spread on banner and grasshoppers fed on the banner margin	**< 5**
Lantana camara L. (Verbenaceae)	A ring-like around the distal portion of corolla tube, at the floral tube entrance	**Beetles** fed on the margins of corolla lobes and the basis of the corolla tube	**< 1**
Lippia alba (Mill.) N.E.Br. ex P. Wilson (Verbenaceae)	Distal portions of corolla lobes, spots on the floral tube entrance, and bracts at the centre of the inflorescence	**Caterpillars** fed on the floral buds covered by bracts at the centre of inflorescence and beetles fed on corolla lobes	**> 30**
Pyrostegia venusta (Ker Gawl.) Miers (Bignoniaceae)	A spot between the two upper corolla lobes	**Caterpillars** fed on the entire flower, depending on its life stage, but they usually fed on the upper portion of the floral tube, including the corolla lobes; grasshoppers and birds remove petal lobes and eventually the upper portion of floral tube	**> 50**
Tocoyena formosa (Cham. & Schltdl.) K.Schum. (Rubiaceae)	The entire corolla, anthers, and stigma margins	**Small beetles** made small holes on petal lobes	**< 3**
Zeyheria montana Mart. (Bignoniaceae)	The margins of corolla lobes, anthers, and stigma	**Beetles**, grasshoppers, and birds remove part of corolla lobes and beetles also made holes on floral tube	**< 20**

We verified that florivory did not affect the total amount of scent emitted by the flowers of seven plant species, regardless of the portions of the flowers that were consumed by the florivores ($p > 0.05$; see the Appendix 2 for detailed statistics for each plant species), including species pollinated by bees (*A. mansoanum*, *B. intermedia* and *C. pubescens*), by butterflies (*L. camara*, *L. alba*) and by hummingbirds (*P. venusta* and *Z. montana*), but affected the total amount of scent emitted by one species, which is pollinated by hawkmoths (*T. formosa*).

DISCUSSION

Osmophores in the studied species fall mainly into the category of diffuse osmophores, but two species, *Lantana camara* and *Pyrostegia venusta*, showed osmophores in well delimited areas. Eight species had variable parts of their osmophores areas removed by florivores, and only one species showed florivory patterns that did not comprise flower areas bearing osmophores. However, contrary to our expectations, in most species florivory did not affect total scent emission, regardless of the type of osmophores presented by plant species and the intensity of floral damage caused by the florivore. The only species that altered scent emission after damage showed the entire lobes, including trichomes, epidermal cells and mesophyll, involved in scent production. Additionally, the florivores were small beetles that made small holes on petal lobes and were not responsible for an expressive reduction in the total area associated with scent emission.

Diffuse osmophores are composed of common epidermal cells and subepidermal parenchyma cells with diffuse distribution throughout the flower such as sepals, petals, and bracts (Fahn, 1979; Vogel, 1983, 1990; Endress, 1994; Caissard *et al*., 2004). In the studied species, cells that encompass osmophores can be recognized by the relatively larger size than adjacent cells, thin cuticle, proportionately large nuclei, dense cytoplasm. Such features are typical of volatile-secreting cells and tissues, as pointed out by Vogel (1990). In addition to typical osmophores, a positive reaction for the presence of volatiles was detected in floral secretory trichomes in several species studied here. Floral secretory trichomes with anatomical and cellular characteristics of osmophores have been described in different families, including Bignoniaceae (Machado *et al*., 2006; Guimarães *et al*., 2008), Leguminosae (Marinho *et al*., 2014, 2016) and Orchidaceae (Vogel, 1990). Terpenes corresponded to the main class of substances detected histochemically in the osmophores and floral secretory trichomes of the studied species, which corresponds to

the major components, being usually responsible for the characteristic floral scent (Vogel, 1983, Knudsen *et al*., 2006; Poser & Mentz, 2007).

The occurrence of osmophores with similar cellular and histochemical characteristics in species from different families, but with animal pollination, is consistent with the role attributed to these glands since floral scent is an important component in plant-pollinator interactions (Dudareva & Pichersky, 2006; Knudsen *et al*., 2006; Wright & Schiestl, 2009). Floral scent has been described as particularly important for bees to locate flowers (Dobson, 1994; Dötterl & Vereecken, 2010). In fact, the three bee-pollinated species *Amphilophium mansoanum*, pollinated by large bees (Yanagizawa & Gottsberger, 1983; Balduino *et al*. *under review*), *Byrsonima intermedia*, pollinated by oil-collecting bees (Boas et al., 2013) and *Centrosema pubescens*, also pollinated by large bees (*pers. obs.*), showed large areas of diffuse osmophores, comprising almost the entire corolla. So, in this pollination-system one could expect that a reduction in scent emission could affect bee's attraction by directly reducing scent release on surrounding air or, even by a reduction in the emission of certain compounds that could attract pollinator bees (García et al. 2021). However, in *A*. *mansoanum* and *C*. *pubescens* the area removed by florivores usually comprised less than 5% of the corolla surface, which could not be enough to represent a significant difference in the total amount of scent emitted by damaged flowers, when compared to undamaged ones. However, in *B*. *intermedia* we registered a pronounced florivory with more than 50% of the flower being consumed by the florivores. Thus, our results suggest that physiological adjusts may occur in this plant species. As we sampled inflorescences and calculated the mean of total amount of scent emitted per flower, one possibility is that intact flowers, in the same inflorescence as those damaged by florivores, start releasing higher amounts of scent as a response triggered by florivory (Kessler & Halitschke, 2009; Zangerl & Berenbaum, 2009), and it compensates the expressive reduction in osmophore portions of damaged flowers.

Lepidopterans are also able to use a variety of chemical cues to locate flowers (Topazzini *et al*., 1990; van Loon *et al*., 1992). Both butterfly-pollinated species, *L. camara* and *L. alba*, showed osmophores surrounding the entrance of corolla tube, which may create a spatial variation in scent emission within the flower (Dötterl & Jürgens, 2005; García et al., 2021) that may act as a chemical floral guide to the hidden nectar. Indeed, this may be important to guide butterflies' visits in these species, as the flowers are disposed in dense inflorescences, with variable degrees of superposition among them. Scented nectar guides are suggested as a trait that shortens the time spent by pollinators

to encounter floral resource (Lawson et al. 2017). *Lantana camara* was the only species that showed no osmophore portions removed by florivores. It might happen due to deterrent compounds present in scent (Kessler and Halitschke 2009 and references therein), or due to physical constraints for the florivores to consume this flower portion. In *L. alba* portions bearing osmophores were consumed by both florivores, caterpillars and beetles, however the entrance of the floral tube was somehow preserved and the osmophores placed there may have a role in maintaining scent emission and, consequently, in maintaining butterfly visitation despite florivores' activity. Several studies have shown that flowers present a greater relative amount of defensive compounds when compared to leaves (Catalfamo et al., 1982; Hartmann et al. 1989; Detzel and Wink 1993; Bravo and Copaja 2002; Frolich et al. 2006; Alves et al., 2007; Beninger et al. 2007; Frolich et al., 2007; Cirak et al. 2008; Zhang et al. 2008). Therefore, it is possible that, in both case in which florivores did not damage osmophores-bearing regions of the corolla, among the volatile compounds emitted by this plant species are some that act as florivore repellents. The only sampled hawkmoth-pollinated species, *Tocoyena formosa*, showed osmophores distributed through the entire corolla. The small damages caused by florivores in strongly scented flowers such as those from *T. formosa*, might suggest that they present deterrent volatile compounds (Kessler and Halitschke 2009 and references therein) or some constitutive defence, such as phenolic compounds (Nakano et al. 2020). However, despite the scarce florivory in this species, the amount of total scent emitted by the flowers was affected, suggesting an indirect response, which may be local or systemic, triggered by florivory (Lucas-Barbosa *et al*., 2013), since the percentage of flower area removed by these small beetles could not explain the significant reduction in the total amount of scent emitted by damaged flowers. Finally, both hummingbird-pollinated species, *P. venusta* and *Z. montana*, showed osmophores portions partially or totally removed by florivores. Hummingbirds have historically been regarded as not presenting a well-developed sense of smell (Bang & Cobb, 1968). However, recent studies have shown that they can detect (Steiger *et al*., 2008; Wester & Lunau, 2017) and react (Kessler et al., 2008) to floral scent, so the importance of the maintenance of scent emission in these plant species cannot be disregarded. Despite florivores having removed most of the flower area bearing osmophores, the total amount of scent emitted by damaged flowers did not differ from that emitted by undamaged flowers, which means that, perceived or not, the total amount of floral scent remained similar in both scenarios.

In synthesis, our findings suggest a resilience in scent emission, since the presence of disturbs of variable intensities and the extension of osmophore area removed by florivores do not seem to matter. The evidence that only one of the sampled plant species showed a florivory-induced reduction in floral scent emission together with the absence of response in the other species may suggest that florivory-induced changes in floral scent are not the norm but are rather a species-specific response. Whereas most plant species adjust their metabolism and continue emitting equivalent amounts of floral scent despite florivory.

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Appendix 1. Details regarding the sample size for each plant species, considering the number of flowers or inflorescences and number of plants sampled, $n_{flowers}$ or $n_{inflorescences}$ (n_{plants}). The species in which we sampled the inflorescence as a whole are indicated by an asterisk.

Plant species	Intact flowers	Flowers damaged by florivores
Amphilophium mansoanum	5 (5)	4 (4)
Byrsonima intermedia	3 (3)	3 (3)
Centrosema pubescens	4 (4)	4 (4)
Lantana camara	2* (2)	2* (2)
Lippia alba	2* (2)	2* (2)
Pyrostegia venusta	5 (5)	5 (5)
Tocoyena formosa	4 (4)	4 (4)
Zeyheria montana	4 (4)	5 (5)

Appendix 2. Detailed statistics of Linear Mixed Models (LMMs) used to evaluate if the total amount of scent emitted by flowers that were fed on by florivores differed from that of intact flowers, following the model: Total amount of scent ~ Treatment + (1|Plant), considering treatment as a fixed factor and plant individual as a random factor.

Amphilophium mansoanum

Random effects

Groups Name	Variance	Std.Dev.
plant (Intercept)	5676	75.34
Residual	19751	140.54

Fixed effects

	Estimate	Std. Error	df	t	p
(Intercept)	397.223	79.331	6.808	5.007	0.00169
Treatment	-118.878	95.438	2.203	-1.246	0.32903

Byrsonima intermedia

Random effects

Groups Name	Variance	Std.Dev.
plant (Intercept)	1173	34.24
Residual	183	13.53

Fixed effects

	Estimate	Std. Error	df	t	p
(Intercept)	44.751	21.258	2.288	2.105	0.154
Treatment	-5.481	11.047	2.000	-0.496	0.669

Centrosema pubescens

Random effects

Groups Name	Variance	Std.Dev.
plant (Intercept)	28572	169.03
Residual	2771	52.64

Fixed effects

	Estimate	Std. Error	df	t	p

(Intercept)	161.896	88.520	3.277	1.829	0.157
Treatment	-40.318	37.223	3.000	-1.083	0.358

Lantana camara

Random effects

Groups Name	Variance	Std.Dev.
plant (Intercept)	8652.9	93.02
Residual	153.1	12.37

Fixed effects

	Estimate	Std. Error	df	t	p
(Intercept)	84.689	66.355	1.018	1.276	0.420
Treatment	-5.487	12.374	1.000	-0.443	0.734

Lippia alba

Random effects

Groups Name	Variance	Std.Dev.
plant (Intercept)	0	0.00
Residual	6066	77.88

Fixed effects

	Estimate	Std. Error	df	t	p
(Intercept)	127.15	55.07	2.00	2.309	0.147
Treatment	46.71	77.88	2.00	0.600	0.610

Pyrostegia venusta

Random effects

Groups Name	Variance	Std.Dev.
plant (Intercept)	847.6	29.11
Residual	8389.0	91.59

Fixed effects

	Estimate	Std. Error	df	t	p
(Intercept)	46.475	42.980	7.933	1.081	0.311

Treatment	43.453	57.928	4.000	0.750	0.495

Tocoyena formosa					
Random effects					
Groups Name	Variance	Std.Dev.			
plant (Intercept)	7655	87.49			
Residual	4328	65.79			
Fixed effects					
	Estimate	Std. Error	df	t	p
(Intercept)	240.816	54.733	4.261	4.400	0.0101
Treatment	197.079	46.518	3.000	4.237	**0.0241**

Zeyheria montana					
Random effects					
Groups Name	Variance	Std.Dev.			
plant (Intercept)	0	0.0			
Residual	17989	134.1			
Fixed effects					
	Estimate	Std. Error	df	t	p
(Intercept)	68.84	54.76	8.00	1.257	0.244
Treatment	18.30	86.58	8.00	0.211	0.838

CAPÍTULO 4

EFEITOS DA FLORIVORIA SOBRE O ODOR FLORAL DE ESPÉCIES VEGETAIS COM DIFERENTES SISTEMAS DE POLINIZAÇÃO

EFEITOS DA FLORIVORIA SOBRE O ODOR FLORAL DE ESPÉCIES VEGETAIS COM DIFERENTES SISTEMAS DE POLINIZAÇÃO

ABSTRACT

Plant-pollinator interactions require the prior establishment of communication pathways, which are modulated by chemical and visual cues. Among them, floral scent is essential to ensure successful pollination in various natural systems. This floral attractant is usually associated with the sensory capacities of each pollinator group. However, such clues can also be used by florivores to locate flowers and their resources of interest. Although both interacting animals are attracted to flowers, their ecological roles can be conflicting. After florivory, flowers may no longer be attractive to pollinators or may even start to repel them, due to changes in floral volatiles. Thus, our objective is to investigate whether florivory induces local changes in the amount and composition of floral scent emitted in plant species with different pollination systems. For this, we characterized and compared the composition of floral volatiles of species pollinated by bees, hummingbirds, hawkmoths and butterflies before and after the flowers are damaged by florivores. We show that only a hawkmoth-pollinated species showed a florivory-induced local decrease in the total amount of scent emitted by its attacked flowers. Moreover, florivory did not affect the relative composition of floral scent in any plant species, regardless of their pollinator group. Thus, florivory did not lead to changes in the chemical traits emitted by attacked flowers of most species, with only one exception. Furthermore, this result indicates that the post-florivory floral scent from most species remains intact, even in the species that showed a reduction in the amount of scent emitted, there was no qualitative change in scent, meaning that pollinators cannot distinguish intact from damaged flowers based on the relative composition of floral scent. Therefore, if floral scent is the main floral attractant used by pollinators to make foraging decisions, this stability in post-florivory relative composition may guarantee the maintenance of pollinator visitation and ensure plant species reproductive success. The set of results from this study puts in question the idea that what is known for herbivory-induced chemical reactions is transposable to florivory-based interactions. This study highlights that florivore-flower-pollinator systems seem to be more stable and less reactive than what we imagined, which could be a result of hundreds of millions of years of these systems maintained in considerable equilibrium.

Keywords: chemical attractants, volatile organic compounds, plant-animal communication, florivory, pollination, chemical signalling.

INTRODUCTION

Pollinator attraction is crucial for plants that depend on biotic vectors for pollen transfer (Willmer, 2011). Plant-pollinator interactions require the prior establishment of a plant-animal communication pathway, which is primarily mediated by chemical and visual cues (Chittka and Thomson, 2001; Schaefer and Ruxton, 2011). Among the floral traits associated with pollination systems, colour and colour pattern (Lunau et al., 2011; Papiorek et al., 2016), as well as odour (Dudareva et al., 2006) and floral shape (Willmer, 2011) are essential to ensure successful pollination, as they are usually associated with the sensory capacities of each group of pollinators (Thomson and Chittka, 2001). However, such clues are used not only by pollinators, but also by florivores to locate flowers and their respective resources of interest (Schaefer and Ruxton, 2011). Although both groups of animals are attracted to flowers, their ecological roles can be conflicting. Pollinators guarantee the reproductive success of plants, by ensuring their sexual reproduction, while florivores can reduce plant reproductive success by feeding on pollen and ovules, or by altering floral cues such as odour, affecting plant-pollinator communication. Such changes in phenotype as a response to florivory can occur directly or indirectly. The first occurs when florivores reduce the physical surface of the flower that is responsible for scent emission and may alter the total amount of scent emitted by flowers (Zangerl & Berenbaum, 2009). And the second occurs when, florivory induces qualitative and/ or quantitative changes in floral scent (Lucas-Barbosa et al., 2013; Farré-Armengol et al., 2015). Additionally, the first type of florivory-induced changes is expressed locally, on the attacked flower itself; whereas the latter changes can be expressed systemically, leading to changes in the scent due to a response of the plant as a whole (Dicke et al., 1990; Turlings and Tumlinson, 1992; Potting et al., 1995; Röse et al., 1996). Thus, if pollinators neglect damaged flowers, plant reproductive success can be negatively affected in both scenarios (Mothershead and Marquis, 2000 and references; Malo et al., 2001). In the latter case, flowers may no longer be attractive to pollinators or may start to repel them, due to changes in floral volatile organic compounds (VOCs) (Zangerl and Berenbaum, 2009).

Most studies carried out so far have focused on the relationship of plants with their mutualists, however, the scientific community's interest in jointly exploring the effects of antagonists on the diversification and evolution of floral characters has recently grown (Caballero et al., 2013 and references).

In this scenario, this study may contribute to an integrated approach on the interference of antagonists on chemical attractants, exploring their effects on the response of pollinators. With respect to post-florivory local scent emission, we expect the volatile compounds released to be 'nonspecific', following the same type of response observed in leaf herbivory, so that there should not be an association between the species' pollination system and the compounds issued after the damage. Therefore, our objective is to investigate, in different pollination systems, whether florivory induces local changes in the total amount and composition of floral scent.

MATERIAL AND METHODS

We characterized the composition of floral volatiles before and after flowers were damaged by florivores and evaluated whether there were any changes in them due to florivory. For this, we first carried out observations on frequency of visits, behaviour, period of visits and abundance, in order to identify the main florivore of each plant species. After obtaining this preliminary information, we collected one to three specimens of florivores and kept them in suitable conditions for use in field experiments.

Initially, we collected volatiles from two intact flowers on the same plant at the time of highest pollinator activity (1st sampling). After that, we place a florivore (at its priority activity time, determined in advance from field observations) in one of the intact flowers. After the florivore fed on the flower, we again collect volatiles from the now damaged flower and also from the other flower that remained intact (control). This second sampling was also carried out at the peak visiting hours of the pollinators, in order to confirm whether potential changes in floral volatiles would be due to damage caused by the florivores or if they would only be due to the passage of time between the first and second sampling (Figure 1).

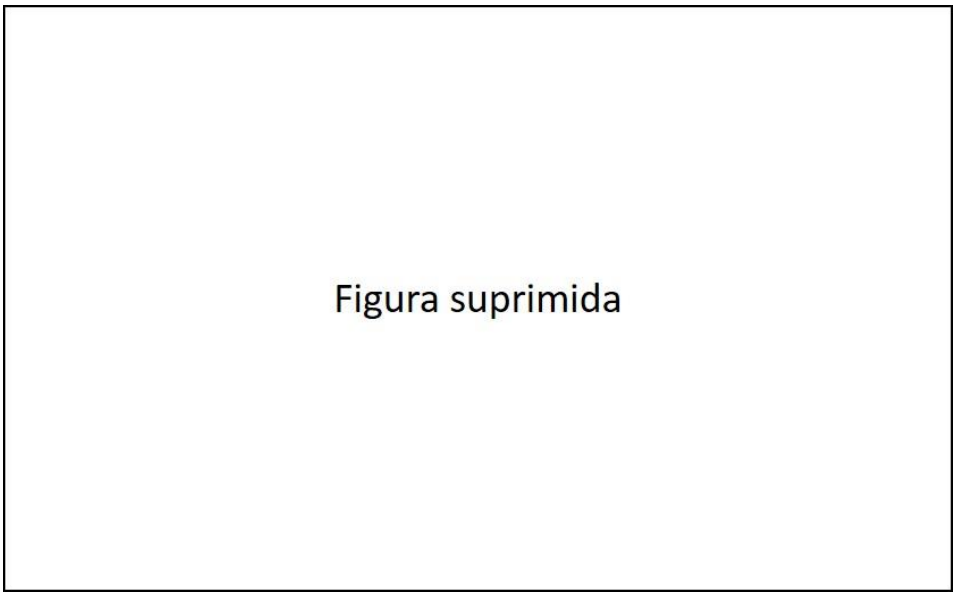

Figure 1. Schematic representation of the experimental design used in the experiments to determine if there are changes in the emission of floral volatiles after the occurrence of florivory. Figure built with Biorender.com.

We collected volatile samples following the protocol by Dötterl et al. (2005). The flowers (or clusters of flowers, in cases of inflorescences with small flowers) were wrapped with polyethylene bags. The volatile compounds accumulated inside the bag were collected in small tubes containing adsorbents, with the aid of a vacuum pump with a flow of 200 ml/min. The duration of collections varied according to the time needed for all the air inside each polyethylene bag to be removed through the pump. In addition, we adapted the protocol for each plant species sampled depending on the specific volatile yield of each one of them. The microtubes, 20 mm in length and 2 mm in internal diameter, were filled with a mixture of 1.5 mg Tenax-TA (60–80 mesh) and 1.5 mg Carbotrap B (20–40 mesh), contained by glass wool. In addition to samples of volatiles collected directly from flowers, we also collect leaf samples to verify unique flower volatiles. The samples were stored in a freezer at approximately -20 °C until the moment of analysis in an automated thermo desorption system attached to a gas chromatograph coupled to a mass spectrometer (GC-MS). This setup was equipped with a ZB-5 fused silica column (60 m long, 0.25 mm of inner diameter, 0.25 μm of film thickness) and maintained a constant 1.5 mL/min flow of helium as the carrier gas. The injector temperature was 200°C and the samples were injected in variable split modes for each species, based on preliminarily runs of test samples of each one of them. The oven temperature started at 40°C, then increased by 6°C/min to 250°C, at which it kept constant

for 1 min. The MS interface was set at 250°C. Mass spectra were taken at 70 eV (in EI mode), with a scanning range of 30–350 m/z. The data were analysed using the GCMSolution package, Version 4.41 (Shimadzu). We identified volatile compounds by comparing their Kovats retention indices (based on a series of n-alkanes) and mass spectra to data available in the databases ADAMS (Adams, 2007), ESSENTIALOILS-23P, FFNSC 2, Wiley 9 and Nist11. We confirmed compound identities by comparing their Kovats retention indices to those of authentic reference standards. For the final identification of compounds, we compared the mass spectrum and Kovat's Retention Index (KI) of the target compounds with authentic standards for each compound. For quantitative analysis of VOCs, we injected 100 ng of each of about 150 compounds, including monoterpenes, aliphatic, and aromatic compounds, into the GC-MS system. We used the mean peak area (total ion current) of these compounds to estimate the total amount of floral odour available in each sample, according to Etl *et al*. (2016).

In order to achieve our goals, we selected seven plant species that include different pollination systems, bees, butterflies, hawkmoths and hummingbirds, and different groups of florivores, caterpillars, beetles and grasshoppers (Table 1, Figure 2).

Table 1. Plant species occurring in cerrado vegetation, type of pollinators and associated florivores. For plant species in which more than one group of florivore fed on the flowers, the florivores indicated in bold were the most common and were employed in the field experiments.

Plant species (Family)	Pollinators	Florivores
Amphilophium mansoanum (DC.) L.G.Lohmann (Bignoniaceae)	Large bees	Beetles
Byrsonima intermedia A. Juss. (Malpighiaceae)	Oil-collecting bees	**Beetles** and caterpillars
Centrosema pubescens Benth. (Leguminosae)	Large bees	**Beetles** and grasshoppers
Lantana camara L. (Verbenaceae)	Butterflies	Beetles
Lippia alba (Mill.) N.E.Br. ex P. Wilson (Verbenaceae)	Butterflies	Beetles and **caterpillars**
Tocoyena formosa (Cham. & Schltdl.) K.Schum. (Rubiaceae)	Hawkmoths	Beetles
Zeyheria montana Mart. (Bignoniaceae)	Hummingbirds	**Grasshoppers** and beetles

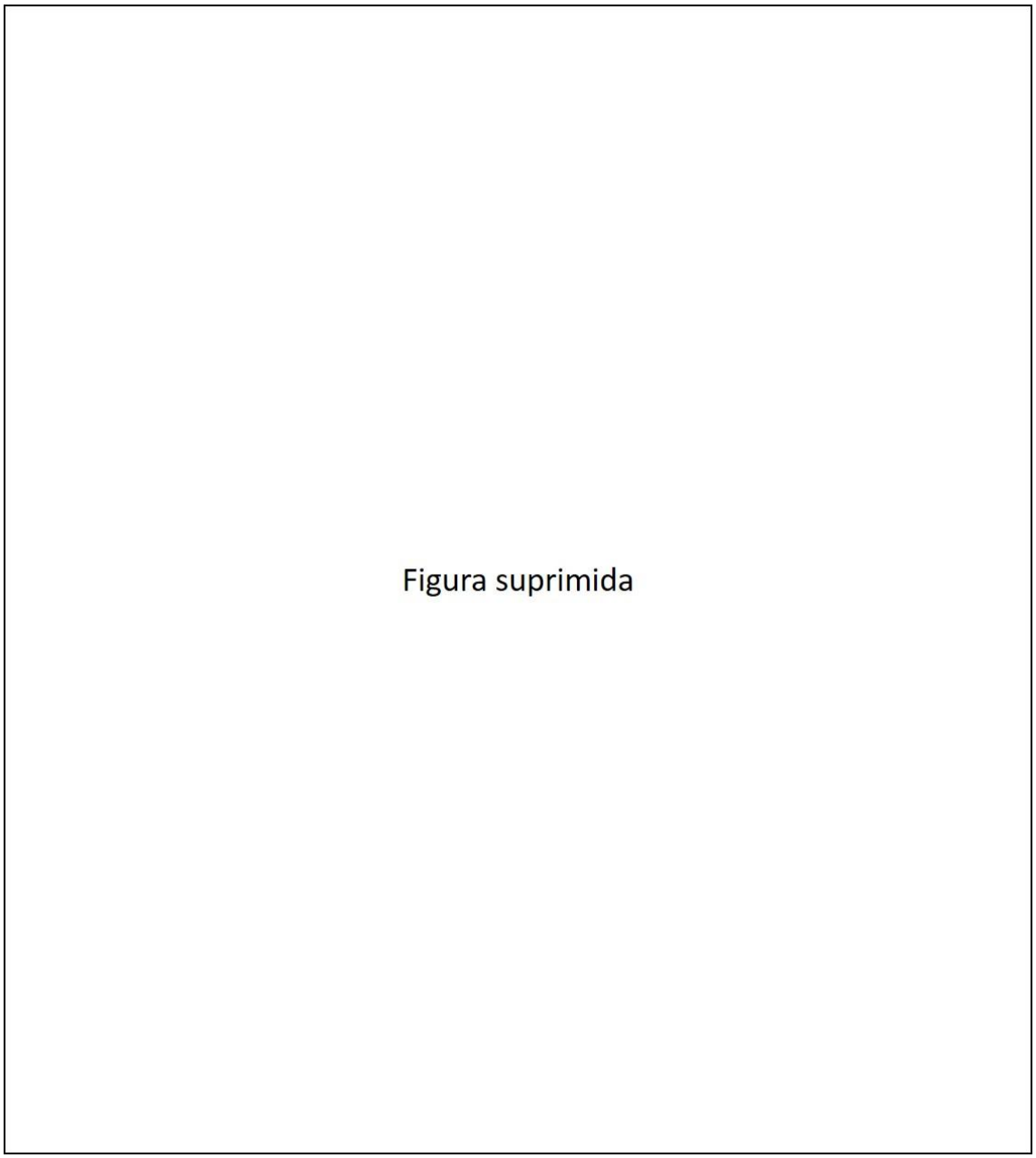

Figure 2. Plant species occurring in cerrado vegetation, type of pollinators and associated florivores. **A.** Flower of *Amphilophium mansoanum* (DC.) L.G. Lohmann (Bignoniaceae) receiving legitimate visit from the pollinating bee *Epicharis flava*. **B.** Damage caused by beetles to the corolla of *A. mansoanum*. **C.** Inflorescence of *Byrsonima intermedia* A. Juss. (Malpighiaceae) receiving legitimate visits from the pollinator oil-collecting bee *Centris* sp. **D.** Caterpillar of the Lycanidae family and damage caused by it to *B. intermedia* flowers. **E.** Flower of *Centrosema pubescens* Benth. (Leguminosae) receiving legitimate visit from the pollinating bee *Bombus* sp. **F.** Grasshopper and damage done by it to a *C. pubescens* flower. **G.** Inflorescences of *Lantana camara* L. (Verbenaceae) receiving legitimate visit from a pollinating butterfly. **H.** Damage caused by beetles to the

corolla of *L. camara*. **I.** Inflorescences of *Lippia alba* (Mill.) N.E.Br. ex P. Wilson (Verbenaceae), which is pollinated by butterflies. **J.** Beetle from the Curculionidae family feeding on *L. alba* flowers. **K.** Flower of the hawkmoth-pollinated *Tocoyena formosa* (Cham. & Schltdl.) K.Schum. (Rubiaceae). **L.** Damage caused by beetles to the corolla of *T. formosa*. **M.** Flowers of *Zeyheria montana* Mart. (Bignoniaceae) receiving a legitimate visit of the hummingbird pollinator *Chlorostilbon lucidus*. **N.** Damage caused by beetles to the corolla of *Z. montana*.

We used PERMANOVA (9999 permutations) to assess whether there are differences between the total amount of volatile compounds emitted by intact and damaged flowers and between the relative amounts of compounds emitted. Based respectively on Euclidean distances and Bray Curtis dissimilarities. For these analyses, we used a factorial sampling design, considering the treatment applied to the flower (intact or consumed by florivores) and the sampling moment (Time 1 or Time 2) as fixed factors and plant individuals as a random factor (Figure 3).

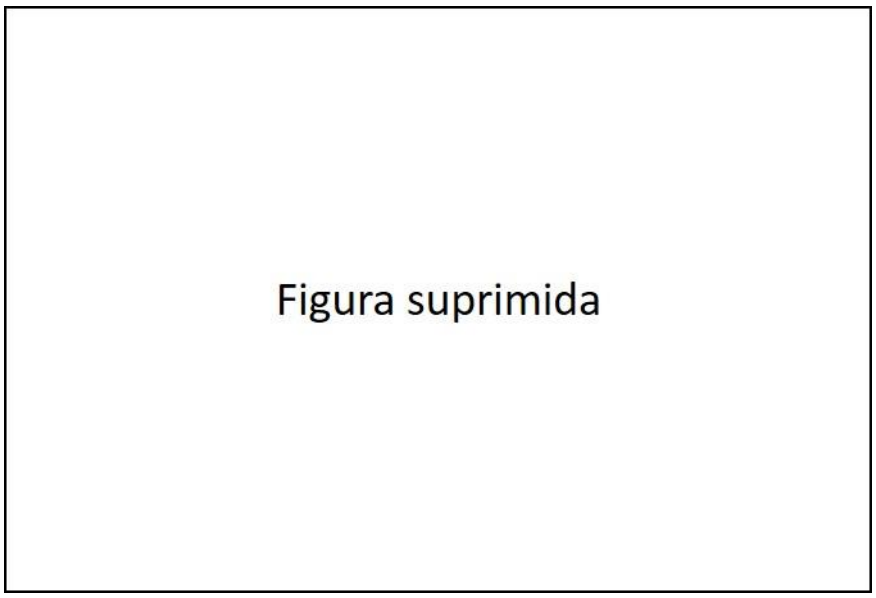

Figure 3. Experimental design used to investigate the emission of floral volatiles in flowers with and without damage caused by florivores. Figure built with Biorender.com.

Moreover, we used non-metric multidimensional scaling (NMDS) to visualize the possible differences between the sampled flowers of each species and between all plant species. For all the PERMANOVAs and NMDSs, we used Primer 6 v. 6.1.15 with PERMANOVA+ v. 1.0.5 (Clarke and Gorley, 2006). To graphically compare the total

amount of volatile compounds emitted by intact and damaged flowers, we also used R v. 4.0.2 (R Core Team, 2020) with additional package ggplot2 (Wickham, 2016).

RESULTS

We verified that florivory did not affect the total amount of emitted volatiles in most of the sampled plant species ($p > 0.05$, Figure 4, Appendix 1, see Appendix 2 for detailed statistics, PERMANOVA 'Treatment*Time'), with the exception of the hawkmoth-pollinated, *Tocoyena formosa*, which showed a significand decrease in the total scent emitted by flowers that were fed on by florivores ($p < 0.05$; Figure 4, see Appendix 3 for detailed statistics).

Moreover, florivory did not affect the relative composition of floral scent in any plant species, i.e. the relative amount of each volatile compound present in floral scent of each plant species ($p > 0.05$, Figure 5, see Appendix 2 for detailed statistics, PERMANOVA 'Treatment*Time').

Additionally, the flowers of the bee-pollinated *Centrosema pubescens* showed a temporal difference in the relative composition of floral scent (Pseudo-F = 4.9876, p = 0.0482, PERMANOVA 'Time').

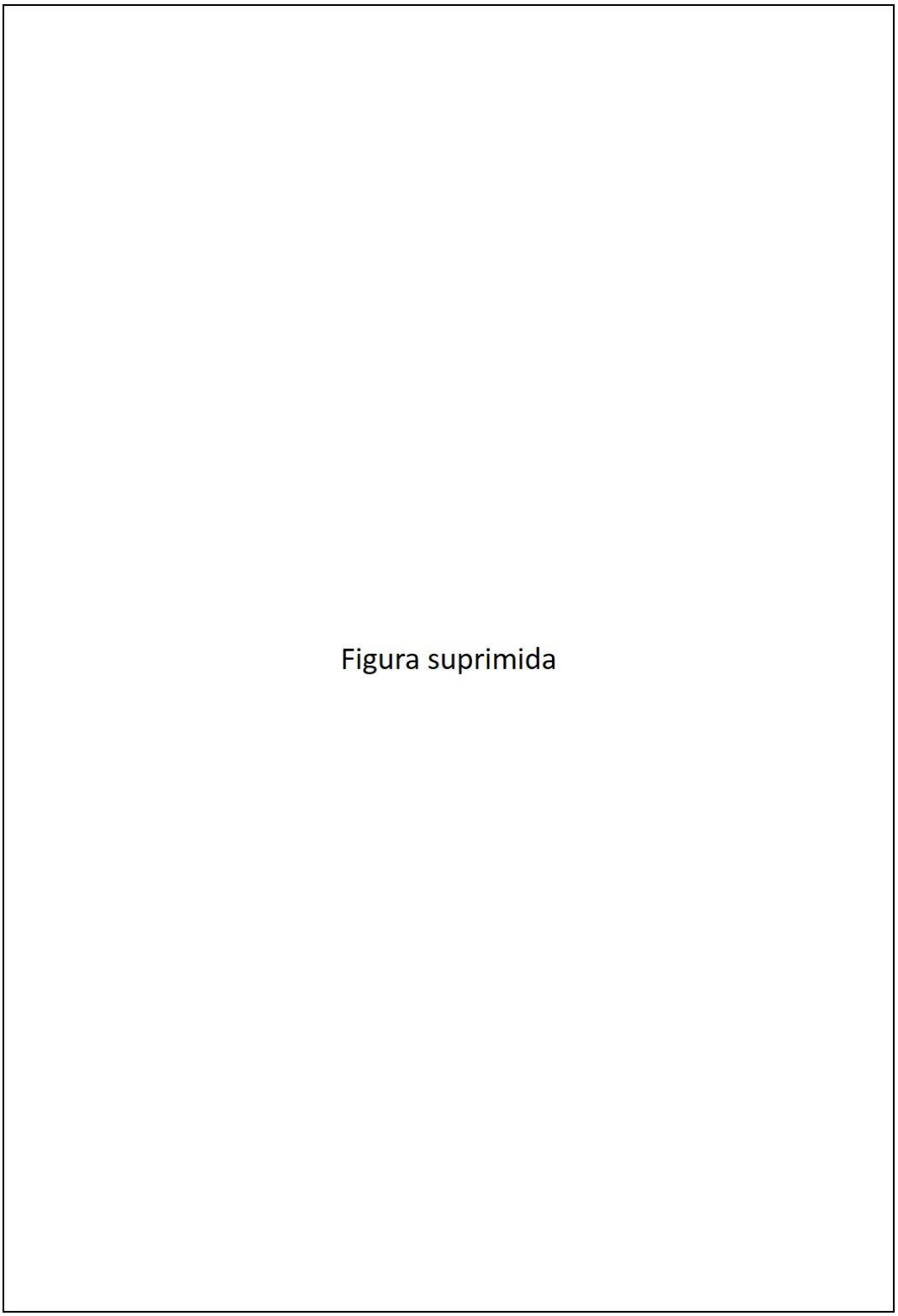

Figure 4. Violin plot of the total amount of scent emitted in flowers of each of the studied plant species (ng.flower^{-1}.hour^{-1} or ng.inflorescence^{-1}.hour^{-1}; see Table 2 for species in which we sampled the whole inflorescence) evincing both treatments ('control' flowers and flowers that were 'fed' on by florivores) and the first and second sampling moments (T1 and T2).

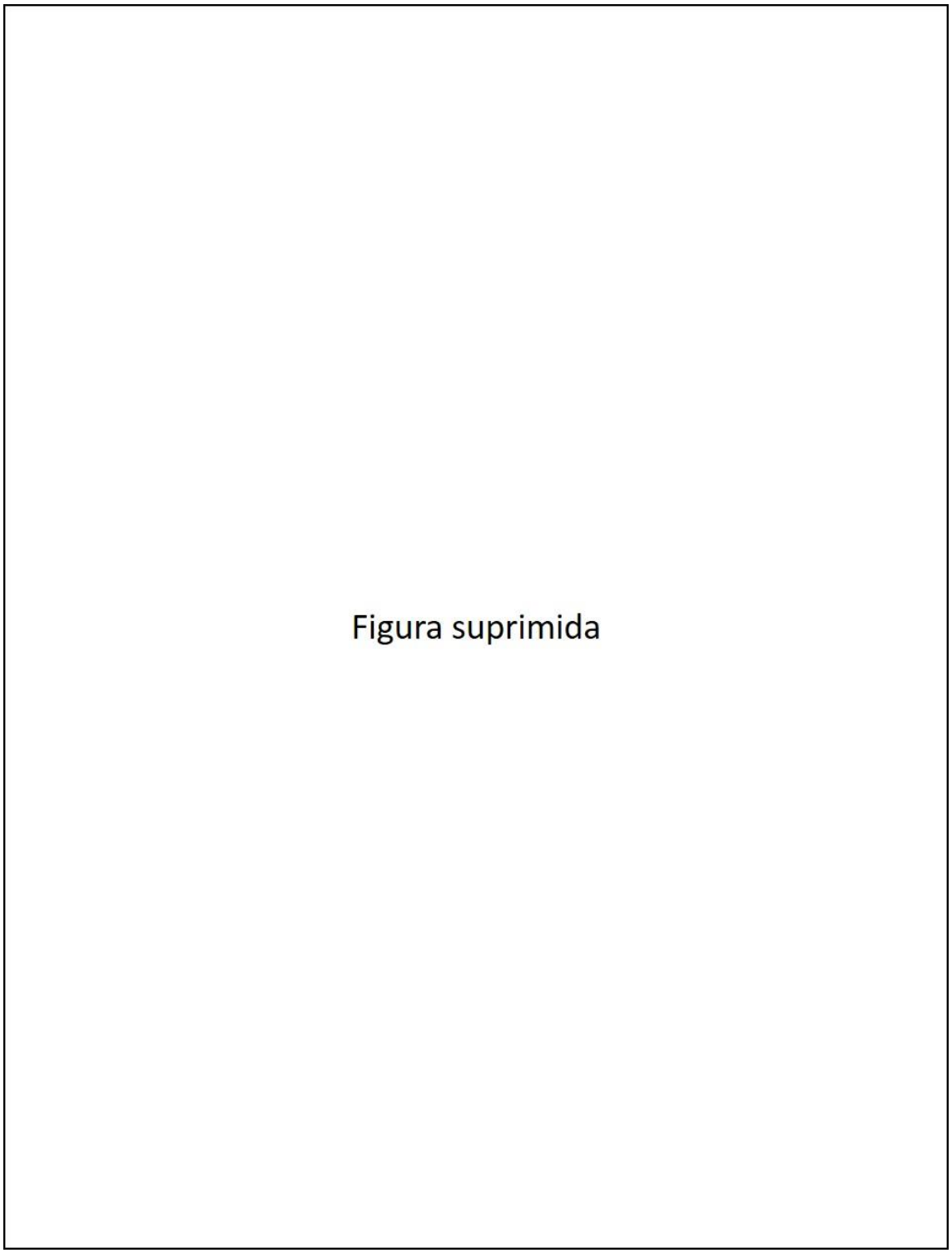

Figure 5. Non-metric multidimensional scaling (NMDS) of floral scent from control and naturally damaged flowers. Comparison between the relative scent composition emitted by flowers that were intact in both Time 1 and Time 2 (control) or flowers that were intact in Time 1, and then were naturally damaged by their respective florivores. The results of each PERMANOVA with 9999 permutations for the 'Treatment'*'Time' interaction are indicated. Each dot represents a sample.

DISCUSSION

In this study, we showed that the evaluated bee-, hummingbird-, butterfly- and hawkmoth- pollinated species produce and emit, mainly terpenes, aliphatic and aromatic compounds. Additionally, we show that the damage caused by florivores to flowers of bee-, hummingbird- and butterfly-pollinated species does not affect the emission of floral volatile organic compounds, neither in terms of total amount nor in terms of its relative composition. Furthermore, we verified that florivory only affected the total amount of scent emitted by *T. formosa*, not affecting its scent relative composition.

Terpenoids constitute the largest and most diverse class of chemical compounds produced as specialized metabolites by plants (McGravey and Croteau, 1995) and are produced through two metabolic pathways, the mevalonic acid pathway and the methylerythritol phosphate pathway (Dudareva et al., 2013). The first being responsible for the synthesis of sesquiterpenes (C15), irregular terpenes and geranyl linalool, and the second responsible for the synthesis of hemiterpenes (C5), monoterpenes (C10), diterpenes (C20) and carotenoid derivatives (C8 - C18) (Dudareva et al., 2013). The presence of terpenes belonging to several of the aforementioned classes shows that the two synthetic pathways are acting in the production of floral volatiles in the studied plant species. Furthermore, the simultaneous presence of several different terpenes in the flower odour of these plant species may be due to the presence of multiproduct terpene synthases, which may be responsible for the co-occurrence of different terpenes in a single plant species (Knudsen and Gershenzon, 2006). In addition to terpenoids, the study species also presented aliphatic compounds, which are produced through the lipoxygenase pathway, and include alcohols, aldehydes and esters (Dudareva et al., 2013 and references). Additionally, these species have aromatic compounds, including benzenes (C6 – C1) and phenylpropanoids (C6 – C3), both classes produced via the Shikimato pathway (Dudareva et al., 2013 and references). Finally, the bee-pollinated, *Centrosema pubescens*, and the hawkmoth-pollinated, *Tocoyena formosa*, also presented volatile compounds containing nitrogen and *Byrsonima intermedia* presented a compound containing Sulphur in its composition, both types of compounds produced by the same metabolic pathway responsible for the synthesis of volatile compounds derived from branched-chain amino acids (Dudareva et al., 2013 and references).

Volatile organic compounds present exclusively in flowers, or floral volatiles, are primarily associated with pollinator attraction (Metcalf and Kogan, 1987; Vogel, 1990; Dobson, 1994). However, these may also be associated with other ecological roles

(Pichersky and Gershenzon, 2002), including acting as a defence against biotic agents. In addition to the natural presence of volatile floral compounds that are repellent to florivores, it is possible that, due to the damage caused by these animals, the affected plants start to emit new volatile compounds or start to produce different amounts of pre-existing compounds, similarly to how different species react after leaf herbivory (Dicke and van Loon, 2000 and references; Holopainen, 2004). Plants are known to present a wide array of herbivore-induced responses, especially regarding plant chemical traits (Kessler & Baldwin 2002; Erb et al.2008; Dicke & Baldwin 2010). The volatile compounds emitted only after damage are termed herbivore-induced plant volatiles (HIPV) (Kessler and Baldwin, 2001; Holopainen, 2004). This term has been used mainly to refer to plant responses to foliar herbivory, however, the same can be applied to florivory. Such HIPV are mainly terpenoids, aliphatic and aromatic compounds (Mumm and Dicke, 2010; Dudareva *et al*., 2004). Therefore, we can infer that the fact that every studied plant species presented the floral volatile biosynthesis pathways that are involved in the synthesis of herbivory-induced volatiles indicates that the absence of chemical response to florivory observed in most plant species is not due to lack of biochemical or cellular machinery in terms of biosynthetic pathways.

Even though most studies focus on the effect of leaf herbivory on leaf or flower volatile emission (Geervliet *et al*., 1994; Turlings & Fritzsche, 1999; Dannon *et al*., 2010), florivores can also affect floral scent (Zangerl & Berenbaum, 2009; Lucas-Barbosa et al., 2013; Farré-Armengol *et al*., 2015). Such changes in phenotype as a response to florivory can occur in two ways, directly by reducing the physical surface of the flower that is responsible for scent emission and altering the total amount of scent emitted by flowers (Zangerl & Berenbaum, 2009), or indirectly by inducing qualitative and/ or quantitative changes in floral scent (Lucas-Barbosa et al., 2013; Farré-Armengol *et al*., 2015). Additionally, the first type of florivory-induced changes are expressed locally, on the attacked flower itself; whereas the latter changes can be expressed systemically, leading to changes in the scent of the attacked plant as a whole (Dicke et al., 1990; Turlings and Tumlinson, 1992; Potting et al., 1995; Röse et al., 1996), which means that even flowers that were not attacked by florivores may start to emit different volatile compounds or different proportions of volatile compounds in its scent when compared to intact flowers on undamaged plants. In the present study, our experimental design allowed us to identify florivory-induced local changes in floral scent as well as potential systemic responses.

In general, the production and emission of floral volatiles are costly in terms of energy for plants (Knudsen and Gershenzon, 2006, Gershenzon, 2017), however, if they provide some type of protection against florivory, for example, their presence may increase the aptitude of the plant species in question, and this can offset the energy cost of their production. Flowers themselves are high-value structures for plant species that depend on biotic cross-pollination to ensure their reproduction (Knudsen and Gershenzon, 2006; Kessler and Halitschke 2009). The 'optimal defence theory' proposes that chemical defences against herbivores are limited and that the distribution of such defences in the plant body is determined by the 'value' of the organ or tissue to the plant, that is, by the contribution of this organ or tissue to the general plant fitness (Rhoades, 1979). Thus, the presence of floral volatiles can provide a double adaptive advantage for plant species if they act simultaneously as attractants for pollinators and repellents from antagonistic animals, such as florivores. Some studies have already shown that flowers have a greater relative amount of defensive compounds when compared to leaves (Catalfamo et al., 1982; Hartmann et al. 1989; Detzel and Wink 1993; Bravo and Copaja 2002; Frolich et al. 2006; Alves et al., 2007; Beninger et al. 2007; Frolich et al., 2007; Cirak et al. 2008; Zhang et al. 2008). For these reasons, we expected an increase in scent emission (ng.flower-1.min-1) in damaged flowers and also a change in its composition in damaged flowers, however, we did not observe an increase in the total amount of scent emitted in any of the studied plant species, contrary to our expectations. Indeed, we even found the opposite response in *T. formosa* flowers, which showed a general reduction of floral scent in attacked flowers without any change in its composition, which is a local response to florivory. Interestingly, the small floral area removed by the florivores of this plant species could not explain the significant reduction in the total amount of scent emitted by attacked flowers, as discussed in Chapter 3 of this thesis. The reduction in scent emission in attacked *T. formosa* flowers could be a physiological adaptation that avoids wasting energy on the production of floral scent in a flower that possibly had its potential for reproduction compromised by florivory. Another possibility is that the reduction of scent emission avoids the infestation by more florivores, possibly making the attacked flowers less conspicuous to florivores.

A 'conflict of interest' commonly occurs in insect-pollinated plants, in which scent may be responsible for attracting both pollinators and flower-eaters (Theis 2006). Furthermore, emitting defensive compounds can lead to a side effect and end up repelling pollinators and not just florivores (Kessler and Halitschke 2009; Kessler 2015). Thus, any

modification in the composition of floral volatiles that could lead to the emission of repellent compounds to florivores, but which in turn were negatively perceived by pollinating insects, could lead to disruption of the interaction between that plant and its pollinators. In this scenario, the lack of changes in scent composition as a response to florivory observed in our study may constitute an advantage for plant species by ensuring that pollinators will continue to perceive flowers in the same way. The only species that showed a change in floral scent relative composition was *C. pubescens*. However, further studies are necessary to determine if such change is due to a natural change in floral scent throughout flower lifespan or if such response is indeed a systemic response to florivory.

The biochemical pathways that lead to the production and release of homoterpenes, such as (E)-4,8-dimethyl-1,3,7-nonatriene, induced by leaf herbivory have already been identified (Donath and Boland, 1994, 1995; Bouwmeester et al., 1999; Degenhardt and Gershenzon, 2000). This compound is known to be released by leaves of several species in response to leaf herbivory (Donath and Boland, 1995) and acts as an attractor of predators of herbivores and parasitic wasps (Dicke et al., 1990; Turlings et al., 1990, 1995; Vet and Dicke, 1992). However, we observed that this compound is naturally produced by flowers of the bee-pollinated species *B. intermedia*, the hummingbird-pollinated *Z. montana*, and the butterfly-pollinated *L. camara* and *L. alba*, which may indicate that this compound can act as a volatile defence compound in these species, even in the absence of any damage done to the flowers.

Flowers pollinated by bees commonly present a floral odour dominated by terpenoids, with few benzenes and few aliphatic compounds (Dobson, 2006), as is the case with the species in the study. Some compounds such as α- and β-pinene, trans-β-ocimene, linalool, limonene, humulene, trans-β-caryophyllene, trans-β-farnesene, α-copaene, caryophyllene, citronellol, germacrene D, myrcene, nerolidol, benzyl alcohol, 1,4-dimethoxybenzene, benzaldehyde, methyl cinnamate, 2-phenylethanol, methyl benzoate, eugenol, among others, are associated with bee pollination (Dobson, 2006). Of these compounds, α- and β-pinene, trans-β-ocimene, linalool, limonene, caryophyllene, myrcene, nerolidol, benzyl alcohol, 2-phenylethanol and eugenol were found in the studied species.

In turn, flowers pollinated by butterflies show abundance of benzenoids, terpenoid, especially *trans*-β-ocimene and linalool (Dobson, 2006), which were found in both butterfly-pollinated plant species studied here, *L. camara* and *L. alba*. Additionally, phenylacetaldehyde and 2-phenylethanol, are also common compounds in the floral scent

of butterfly-pollinated species. These scent compounds may act as signals to butterflies (Dobson, 2006), especially phenylacetaldehyde to which Glossata species show a strong specificity towards (Guo et al., 2021). However, neither of them is present in the studied species.

Floral scent of hawkmoth-pollinated plants usually shows acyclic terpene alcohols, such as linalool, nerolidol and farnesol, simple aromatic compounds, such as methyl benzoate, benzyl acetate, methyl salicylate, benzyl alcohol and 2-phenylethanol, nitrogen-bearing compounds, such as indole, oximes and methyl anthranilate, also jasmonates, tiglates, and lactones; and may also contain trans-ocimene, citronellol and geraniol (Dobson, 2006). From these compounds, we found methyl benzoate, methyl salicylate, 2-phenylethanol, and the N-bearing, 2-methylbutanenitrile.

Hummingbird-pollinated plant species present highly variable floral scent (Dellinger *et al*., 2019; Tunes *et al*., under review, Chapter 5 of this thesis), being that some plant species do not show any scent on their flowers (Knudsen *et al*., 2004). Even though it is not unusual for hummingbird-pollinated plant species to present scentless flowers, hummingbirds can detect (Steiger *et al*., 2008; Wester & Lunau, 2017) and react (Kessler et al., 2008) to floral scent. Therefore, if florivory-induced changes in floral scent were to occur in these species, it is possible that it would affect hummingbird visitation.

Little is known about the role of individual compounds in plant-animal interactions, whether mutualistic or antagonistic, especially under natural conditions (Dudareva *et al*., 2006). Flowers usually present a set of multisensory attractants that can be used together by animals to locate flowers in the environment, however, the floral scent is the one most easily learned and memorized by bees (Wright and Schiestl 2009, Lawson et al. 2018). In the present study, we verified that florivory only affected the total amount of scent emitted by the flowers of a single plant species. This indicates that most plants do not have specific defences induced by the action of these antagonists, which suggests that florivory-induced chemical responses are highly species-specific. Furthermore, this result indicates that, even after damage caused by florivores, the chemical signalling of flowers of most species remains intact, even in the plant species that showed a reduction in the amount of scent emitted, there was no qualitative change in scent, which means that pollinators cannot chemically distinguish intact from damaged flowers, maintaining their visitation and ensuring the reproductive success of plant species. The results of the present study put in question the idea that what is known for herbivory is transposable to

florivory-based interaction. Therefore, this study raises more questions regarding florivore-flower-pollinator systems and highlights that systems involving florivory seem to be more stable and less reactive than what we imagined, which could be a result of hundreds of millions of years of these systems in considerable equilibrium.

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Appendix 1. Total absolute (mean ± sd; ng.flower^{-1}.hour^{-1} or ng.inflorescence^{-1}.hour^{-1}) and relative amount (mean ± sd; minimum-maximum; %) of scent emitted by flowers of each plant species submitted to each treatment. Species for which scent is presented for the inflorescence are indicated by +. Scent compounds are listed according to their chemical class and Kovats' Retention Index (RI) based on a series of n-alkanes (C6-C20). Compounds marked by an asterisk were identified based on their mass spectra and the RI of synthetic standards. T1 = Time 1; T2 = Time 2; tr = trace amount (mean <0.1%).

Bee-pollinated plant species	RI	*A. mansoanum*				*B. intermedia*				*C. pubescens*			
		Control		Fed		Control		Fed		Control		Fed	
		T1	T2	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Total number of compounds		41	30	43	34	47	43	43	44	52	37	51	40
Nsamples (Nplants)		6 (5)	5 (5)	6 (5)	5 (5)	4 (4)	3 (3)	4 (4)	3 (3)	4 (4)	4 (4)	4 (4)	4 (4)
Total amount of scent emitted (ng/ flower/ h)		497.8 ± 127.2	278.3 ± 177.2	491.4 ± 298.0	444.5 ± 135.6	44.3 ± 50.8	39.4 ± 34.9	19.9 ± 19.3	34.1 ± 38.5	170.9 ± 83.0	122.1 ± 138.9	144.0 ± 134.0	162.6 ± 209.7
Alifatic compounds													
4-Hexen-1-yl ester butanoic acid	646	-	-	-	-	tr	0.5 ± 0.9 (0-1.6)	0.7 ± 1.4 (0-2.9)	0.4 ± 0.7 (0-1.3)	-	-	-	-
2-Butenal	646	-	-	0.6 ± 1.6 (0-4.1)	0.8 ± 1.8 (0-4.1)	-	-	-	-	-	-	-	-
2-Butanyl acetate	752	-	-	0.6 ± 1.5 (0-3.6)	0.7 ± 1.6 (0-3.6)	-	-	-	-	-	-	-	-
4-Methyl-3-pentene-2-ol*	793	-	-	-	-	-	-	-	-	0.1 ± 0.3 (0-0.6)	0.8 ± 0.9 (0-2.1)	0.2 ± 0.4 (0-0.8)	0.9 ± 1.2 (0-2.6)
Hexanal*	796	0.5 ± 0.9 (0-2.3)	-	1.8 ± 3.8 (0-9.4)	0.5 ± 0.7 (0-1.7)	2.1 ± 3.3 (0-7.1)	-	0.9 ± 1.4 (0-3.0)	-	-	-	-	-
(Z)-2-Hexenal*	852	-	-	-	-	0.4 ± 0.8 (0-1.6)	-	tr	-	-	-	-	-
1-Methoxy-2-propyl acetate	866	-	-	-	-	3.1 ± 6.3 (0-12.6)	-	6.1 ± 12.3 (0-24.7)	-	-	-	-	-
Methyl hexanoate*	922	tr	0.1 ± 0.2 (0-0.5)	tr	-	-	-	-	-	-	-	-	-
α-2-Methyl-2-vinyl-5-hydroxytetrahydrofuran	928	-	-	-	-	-	-	-	-	0.2 ± 0.3 (0-0.6)	0.3 ± 0.5 (0-1.1)	0.2 ± 0.5 (0-1.1)	0.1 ± 0.1 (0-0.4)
2,2-Dimethyl-1,3-propanediol	929	-	-	-	-	-	tr	tr	tr	-	-	-	-

2-Methyl-2-vinyl-5-hydroxytetrahydrofuran	946	-	-	-	-	-	-	-	-	0.1 ± 0.1 (0-0.3)	0.1 ± 0.3 (0-0.6)	0.2 ± 0.4 (0-0.8)	tr
2,3-Dimethyl-1-butanol	954	-	-	-	-	-	-	-	-	tr	-	tr	-
1-Decanol	958	-	-	-	-	tr	tr	tr	tr	-	-	-	-
(2-Phenyl-1,3-dioxolan-4-yl)methyl ester octadecanoic acid	970	-	-	-	-	-	tr	-	tr	-	-	-	-
6-Acetoxy-p-menta-1,8-diene	1012	-	-	-	-	-	tr	-	tr	-	-	-	-
1-Decanol	1060	-	-	-	-	0.3 ± 0.7 (0-1.4)	-	tr	-	-	-	-	-
Methyl octanoate*	1120	-	-	-	-	0.2 ± 0.4 (0-0.9)	-	tr	-	-	-	-	-
(Z)-3-Hexenyl butyrate*	1188	-	-	-	-	-	-	-	-	tr	0.2 ± 0.5 (0-1.1)	tr	0.5 ± 1.1 (0-2.3)
2,6-Dimethyl-1,5-Z,7-octatrien-3-ol	1187	0.1 ± 0.3 (0-0.9)	0.1 ± 0.1 (0-0.3)	0.1 ± 0.2 (0-0.5)	-	-	-	-	-	-	-	-	-
3,7-Dimethyl-1,5-octadiene-3,7-diol	1188	-	0.1 ± 0.2 (0-0.5)	tr	0.7 ± 1.2 (0-2.9)	-	-	-	-	0.1 ± 0.1 (0-0.2)	0.1 ± 0.2 (0-0.4)	0.2 ± 0.3 (0-0.7)	0.3 ± 0.4 (0-0.9)
Ethyl octanoate	1195	0.9 ± 2.2 (0-5.6)	-	1.3 ± 3.2 (0-8)	-	-	-	-	-	-	-	-	-
4-Oxononanal	1249	-	-	-	-	tr	tr	tr	tr	-	-	-	-
3,7-Dimethyl-octa-1,7-dien-3,6-diol	1273	-	0.2 ± 0.4 (0-1.1)	tr	0.4 ± 0.6 (0-1.4)	0.3 ± 0.7 (0-1.4)	-	tr	-	0.6 ± 0.6 (0-1.3)	0.1 ± 0.1 (0-0.4)	0.3 ± 0.4 (0-1)	0.5 ± 0.6 (0-1.4)
Methyl decanoate	1322	-	-	-	-	0.3 ± 0.5 (0-1.1)	10.3 ± 17.9 (0-31.0)	1.4 ± 2.5 (0-5.1)	tr	tr	-	-	0.1 ± 0.1 (0-0.4)
Tetradecane	1401	-	-	-	-	0.1 ± 0.2 (0-0.4)	0.2 ± 0.4 (0-0.8)	-	0.4 ± 0.7 (0-1.2)	-	-	-	-
Dihydro-ß-Ionone*	1452	1.4 ± 1.5 (0-3.4)	0.1 ± 0.2 (0-0.5)	1.5 ± 2.2 (0-6.0)	0.3 ± 0.5 (0-1.1)	-	-	-	-	-	-	-	-
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	1649	-	-	-	-	tr	tr	tr	tr	-	-	-	-
Methyl tetradecanoate	1723	-	-	-	-	-	-	-	-	tr	tr	tr	3.6 ± 6.8 (0-13.9)
Methyl pentadecanoate	1824	-	-	-	-	-	-	-	-	tr	-	-	tr
Aromatic compounds													
Benzaldehyde*	963	-	-	-	-	-	tr	-	0.1 ± 0.2 (0-0.4)	-	-	-	-
Benzyl alcohol*	1036	-	-	-	-	-	tr	-	tr	1.0 ± 0.7 (0-1.6)	0.1 ± 0.2 (0-0.4)	1.7 ± 1.8 (0-4.3)	0.1 ± 0.2 (0-0.5)

Methyl phenethyl ether	1086	-	-	-	-	-	-	-	-	tr	tr	tr	tr
2-Phenylethanol*	1117	0.6 ± 0.6 (0-1.4)	0.2 ± 0.3 (0-0.7)	0.5 ± 0.5 (0-1.3)	-	-	-	-	-	63.9 ± 21.3 (33.1-79.8)	32.9 ± 13.7 (16.8-50.3)	54.0 ± 19.9 (31.6-75.3)	34.2 ± 18.4 (10.5-55.5)
2-Phenylethyl formate*	1180	-	-	-	-	-	-	-	-	tr	-	tr	-
Methyl salicylate	1204	-	-	-	-	0.1 ± 0.3 (0-0.6)	-	0.1 ± 0.2 (0-0.5)	-	-	-	-	-
para-Anisaldehyde*	1263	-	-	-	-	0.3 ± 0.6 (0-1.2)	-	0.3 ± 0.7 (0-1.5)	-	tr	0.3 ± 0.5 (0-1.2)	tr	0.1 ± 0.2 (0-0.4)
para-Anisyl alcohol*	1288	-	-	-	-	-	-	-	-	-	tr	-	tr
para-Propylanisole	1307	-	-	-	-	-	-	-	-	tr	0.2 ± 0.4 (0-0.8)	tr	0.1 ± 0.3 (0-0.7)
Anisyl formate	1341	-	-	-	-	-	-	-	-	0.1 ± 0.1 (0-0.2)	0.4 ± 0.5 (0-1.2)	tr	0.2 ± 0.3 (0-0.6)
Eugenol*	1366	0.1 ± 0.3 (0-0.8)	tr	0.3 ± 0.4 (0-1.1)	0.1 ± 0.2 (0-0.5)	-	-	-	-	-	-	-	-
4-Methoxyphenylethylalcohol*	1375	-	-	-	-	-	-	-	-	8.9 ± 3.7 (4.7-13.7)	32.2 ± 17 (15.8-49.6)	3.5 ± 3.2 (0-7.4)	21.7 ± 15.9 (4.9-39.3)
(E)-Methylcinnamate*	1392	-	-	-	-	-	-	-	-	-	tr	0.3 ± 0.7 (0-1.5)	-
Benzyl 2-methylbutanoate	1394	-	-	-	-	-	-	-	-	tr	-	tr	-
Benzyl isovalerate*	1400	-	-	-	-	-	-	-	-	tr	0.3 ± 0.7 (0-1.4)	tr	tr
Benzyl tiglate*	1506	-	-	-	-	-	-	-	-	0.2 ± 0.4 (0-0.9)	-	tr	-
4-Methoxyphenylethyl acetate	1514	-	-	-	-	-	-	-	-	tr	0.1 ± 0.2 (0-0.4)	tr	0.2 ± 0.4 (0-0.8)
N-bearing compounds													
Phenylacetonitrile*	1145	-	-	-	-	0.7 ± 1.0 (0-2.2)	1.0 ± 1.3 (0-2.6)	1.6 ± 2.4 (0-5.2)	0.7 ± 0.7 (0-1.4)	-	-	-	-
3-Phenylpropanenitrile	1246	-	-	-	-	-	-	-	-	tr	-	tr	-
Indol*	1303	tr	-	tr	1.7 ± 3.9 (0-8.7)	tr	tr	-	tr	tr	-	tr	-
S-bearing compounds													
Dimethyldisulfide*	741	-	-	-	-	-	0.2 ± 0.3 (0-0.6)	0.2 ± 0.5 (0-1)	0.2 ± 0.4 (0-0.7)	-	-	-	-
Terpenoids													

α-Pinene*	939	-	-	-	0.1 ± 0.2 (0-0.5)	-	tr	tr	tr	-	-	-	-
(+)-β-Citronellene*	946	-	-	-	-	-	tr	tr	tr	-	-	-	-
β-Pinene*	979	0.1 ± 0.2 (0-0.7)	-	0.1 ± 0.4 (0-1.0)	-	-	-	-	-	-	-	-	-
6-Methyl-5-hepten-2-one*	987	-	-	-	-	-	0.3 ± 0.6 (0-1.1)	-	0.9 ± 1.6 (0-2.9)	-	-	-	-
β-Myrcene*	990	1.5 ± 1.2 (0-2.8)	0.7 ± 1.0 (0-2)	0.8 ± 1.0 (0-2.2)	2.3 ± 2.6 (0-6.3)	-	-	-	-	0.2 ± 0.1 (0-0.4)	0.4 ± 0.5 (0-1.1)	2.0 ± 2.1 (0.1-4.9)	0.2 ± 0.2 (0-0.6)
δ-3-Carene*	1008	-	-	-	-	-	-	-	-	tr	-	tr	-
Limonene*	1036	-	-	-	-	-	-	-	-	tr	-	tr	-
(Z)-β-Ocimene*	1037	9.6 ± 1.9 (7.3-12.5)	5.9 ± 4.2 (0-10.5)	11.1 ± 4.8 (6.5-18.1)	8.3 ± 6.1 (3.1-18)	0.4 ± 0.8 (0-1.7)	-	0.1 ± 0.2 (0-0.5)	-	0.3 ± 0.5 (0-1.1)	-	0.9 ± 1.0 (0-2.4)	-
Eucalyptol*	1040	-	-	-	-	tr	tr	tr	tr	-	-	-	-
Lavender lactone*	1044	-	-	-	-	-	-	-	-	0.1 ± 0.2 (0-0.5)	0.3 ± 0.5 (0-1.1)	0.1 ± 0.3 (0-0.6)	0.3 ± 0.3 (0-0.8)
(E)-β-Ocimene*	1049	64.9 ± 2.5 (61.7-68.3)	51.7 ± 18.0 (32.9-76.2)	56.2 ± 15.4 (29.9-74.0)	54.2 ± 17.9 (30-73.8)	1.6 ± 3.3 (0-6.6)	0.4 ± 0.8 (0-1.4)	0.9 ± 1.8 (0-3.6)	0.2 ± 0.4 (0-0.7)	2.5 ± 4.9 (0-9.9)	0.1 ± 0.3 (0-0.6)	8.4 ± 9.9 (0-21.2)	0.5 ± 0.7 (0-1.5)
γ-Terpinene*	1063	-	-	-	-	-	-	-	-	tr	-	tr	-
(Z)-Linalool oxide (furanoid)*	1076	tr	-	-	0.4 ± 1.1 (0-2.4)	0.2 ± 0.5 (0-1.1)	tr	0.1 ± 0.2 (0-0.5)	tr	0.2 ± 0.2 (0-0.5)	0.6 ± 0.6 (0-1.5)	0.2 ± 0.3 (0-0.5)	0.4 ± 0.3 (0-0.9)
(E)-Linalool oxide (furanoid)*	1092	-	0.1 ± 0.3 (0-0.7)	tr	0.7 ± 1.0 (0-2.4)	tr	-	tr	-	0.2 ± 0.2 (0-0.6)	0.3 ± 0.3 (0-0.8)	0.1 ± 0.1 (0-0.3)	0.2 ± 0.2 (0-0.6)
α-Terpinolene*	1094	-	-	-	-	-	-	-	-	tr	-	tr	-
(Z)-4,8-Dimethyl-1,3,7-nonatriene	1099	-	-	-	-	0.3 ± 0.7 (0-1.5)	-	0.1 ± 0.2 (0-0.4)	-	-	-	-	-
Linalool*	1099	1.3 ± 3.3 (0-8.1)	20.5 ± 27.6 (0-67)	2.1 ± 2.6 (0-6.9)	15 ± 19.5 (0-40.7)	1.7 ± 3.5 (0-7)	0.1 ± 0.2 (0-0.3)	0.6 ± 1.2 (0-2.5)	tr	16.5 ± 19.1 (0.07-44.1)	24.9 ± 21.2 (0-45.5)	23.3 ± 16.9 (0.2-36.3)	32.0 ± 22.3 (0-49.8)
Hotrienol	1104	-	-	-	-	-	-	-	-	-	0.1 ± 0.3 (0-0.6)	-	0.1 ± 0.3 (0-0.6)
(E)-4,8-dimethyl-1,3,7-nonatriene*	1119	-	-	-	-	2.3 ± 4.6 (0-9.4)	tr	1.0 ± 2.1 (0-4.2)	tr	-	-	-	-
Ocimene derivative	1125	0.2 ± 0.3 (0-0.7)	3 ± 5.8 (0-13.4)	0.8 ± 1.1 (0-2.8)	0.3 ± 0.3 (0-0.6)	-	tr	-	tr	-	-	-	-
Allo-Ocimene*	1130	1.3 ± 1.6 (0-3.3)	3.2 ± 3.7 (0-8.1)	1.9 ± 1.6 (0-3.2)	2.1 ± 1.4 (0-3.8)	0.2 ± 0.4 (0-0.8)	tr	tr	tr	0.1 ± 0.2 (0-0.4)	-	0.1 ± 0.08 (0-0.2)	tr
para-1,3,8-Menthatriene	1134	5.4 ± 4.0 (0-9.6)	5.6 ± 3.9 (0-10.2)	6.8 ± 7.6 (0-17.8)	3.3 ± 2.4 (0-5.9)	0.2 ± 0.4 (0-0.8)	0.1 ± 0.1 (0-0.3)	0.1 ± 0.2 (0-0.5)	tr	0.2 ± 0.4 (0-0.8)	tr	0.3 ± 0.3 (0-0.6)	tr

(E)-Ocimene epoxide*	1143	tr	-	tr	0.1 ± 0.1 (0-0.2)	-	-	-	-	-	-	-	-
Neo-allo-Ocimene*	1145	tr	tr	0.1 ± 0.3 (0-0.7)	-	-	-	-	-	-	-	tr	-
1,2-Dimethoxybenzene*	1145	-	-	-	-	-	-	-	-	2.0 ± 1.1 (0.9-3.6)	1.1 ± 0.9 (0-2.1)	1.3 ± 0.8 (0.7-2.5)	1.0 ± 0.8 (0-2.0)
(Z)-linalool oxide (pyranoid)	1175	-	-	-	-	0.1 ± 0.2 (0-0.5)	tr	-	tr	tr	tr	tr	0.1 ± 0.1 (0-0.2)
(E)-linalool oxide (pyranoid)*	1175	-	-	-	-	-	-	-	-	tr	tr	tr	0.1 ± 0.1 (0-0.4)
α-Terpineol	1198	-	-	-	-	-	-	-	-	tr	tr	tr	tr
para-Methoxyphenylethanol	1250	-	-	-	-	-	-	-	-	-	tr	-	tr
Geraniol	1255	-	-	-	-	-	-	-	-	tr	-	tr	-
2-Phenylethyl acetate*	1262	-	-	-	-	-	-	-	-	0.2 ± 0.2 (0-0.5)	-	tr	-
Neodihydrocarveol	1286	-	-	-	-	0.1 ± 0.2 (0-0.5)	-	0.4 ± 0.9 (0-1.8)	-	-	-	-	-
(Z)-Dihydro-carveol	1287	-	-	-	-	tr	tr	-	tr	-	-	-	-
Lavandulyl acetate*	1290	-	-	-	-	0.1 ± 0.2 (0-0.5)	-	tr	-	-	-	-	-
Chrysanthenone	1317	-	-	-	-	tr	tr	-	tr	-	-	-	-
β-Bourbonene*	1407	-	tr	tr	-	-	-	-	-	-	-	-	-
β-Elemene	1408	-	-	-	-	tr	0.1 ± 0.2 (0-0.4)	tr	0.1 ± 0.1 (0-0.3)	-	-	-	-
β-Caryophyllene*	1444	0.1 ± 0.2 (0-0.4)	0.2 ± 0.3 (0-0.7)	tr	tr	70.9 ± 42.7 (6.9-94.7)	58.7 ± 50.8 (0-89.7)	69.2 ± 45.1 (1.5-94.1)	90.9 ± 4.9 (87.4-96.6)	-	-	-	-
2-epi-(E)-β-Caryophyllene	1454	-	-	-	-	tr	0.5 ± 0.5 (0-1)	-	0.4 ± 0.7 (0-1.3)	-	-	-	-
(E)-Caryophyllene	1474	-	-	-	-	tr	tr	-	tr	-	-	-	-
α-Caryophyllene*	1481	-	-	-	-	10.2 ± 13.1 (3.5-29.9)	25.8 ± 37.3 (4.1-68.9)	4.2 ± 4.6 (0-10.8)	3.9 ± 0.6 (3.2-4.4)	-	-	-	-
β-Ionone*	1499	2.9 ± 2.3 (0-6.7)	0.8 ± 1.1 (0-2.7)	2.1 ± 2.5 (0-6.8)	1.3 ± 1.6 (0-3.2)	-	-	-	-	tr	tr	tr	tr
Germacrene D*	1497	-	-	-	-	-	tr	-	tr	-	-	-	-
(E,E)-α-Farnesene*	1512	0.1 ± 0.1 (0-0.4)	tr	tr	0.4 ± 0.7 (0-1.7)	-	-	-	-	-	-	-	-

Nerolidol	1536	-	-	-	-	0.4 ± 0.8 (0-1.8)	tr	0.1 ± 0.2 (0-0.4)	tr	-	-	-	-
Dihydroisocaryophyllene epoxide	1567	-	-	-	-	0.2 ± 0.2 (0-0.6)	tr	0.4 ± 0.8 (0-1.8)	tr	-	-	-	-
Caryophyllene oxide derivative	1582	-	-	-	-	tr	tr	-	tr	-	-	-	-
Caryophylla-3(15),7(14)-dien-6-ol	1664	-	-	-	-	0.7 ± 1.3 (0-2.7)	tr	tr	tr	-	-	-	-
Neoiso-isopulegol	1667	-	-	-	-	tr	-	tr	-	-	-	-	-
Kessane	1705	-	-	-	-	tr	tr	9.6 ± 19.2 (0-38.5)	0.1 ± 0.1 (0-0.2)	-	-	-	-
Benzyl benzoate*	1785	-	-	-	-	-	-	-	-	tr	-	tr	-
Unidentified compounds													
m/z: 43,57,84,55,71	779	-	-	-	-	-	-	-	-	tr	tr	tr	tr
m/z: 95,67,41,53,81	890	0.1 ± 0.3 (0-0.8)	0.3 ± 0.4 (0-1)	0.7 ± 0.6 (0-1.5)	-	-	-	-	-	-	-	-	-
m/z: 43,70,57,84,96	902	-	-	-	-	tr	-	tr	-	-	-	-	-
m/z: 55,70,94,83,43	977	0.6 ± 1.5 (0-3.7)	-	1.6 ± 3.9 (0-9.6)	-	-	-	-	-	-	-	-	-
m/z: 57,43,105,71,85	999	0.1 ± 0.3 (0-0.7)	-	0.1 ± 0.2 (0-0.6)	-	-	-	-	-	-	-	-	-
m/z: 91,119,134,77,105	1010	1.1 ± 1.2 (0-2.7)	1.5 ± 1.5 (0-3.3)	2.2 ± 3.1 (0-7.9)	1.1 ± 0.7 (0.3-2.2)	-	-	-	-	-	-	-	-
m/z: 81,41,69,79,150	1015	-	-	-	-	-	-	-	-	0.2 ± 0.1 (0-0.4)	0.2 ± 0.1 (0-0.4)	0.3 ± 0.2 (0-0.5)	0.2 ± 0.1 (0-0.4)
m/z: 81,44,79,53,60	1023	tr	-	tr	tr	-	-	-	-	-	-	-	-
m/z: 77,57,43,107,121	1026	-	-	-	-	tr	-	tr	-	-	-	-	-
m/z: 69,55,41,109,85	1079	tr	tr	tr	tr	-	-	-	-	-	-	-	-
m/z: 91,119,134,41,77	1082	0.4 ± 0.4 (0-0.9)	0.2 ± 0.3 (0-0.7)	0.5 ± 0.5 (0-1.5)	0.2 ± 0.2 (0-0.5)	-	-	-	tr	-	-	-	-
m/z: 55,70,41,83,119	1163	0.8 ± 0.8 (0-2)	0.5 ± 0.9 (0-2)	0.9 ± 1.2 (0-3)	0.4 ± 0.4 (0-0.9)	-	-	-	-	-	-	-	-
m/z: 95,93,41,123,150	1168	0.1 ± 0.2 (0-0.5)	-	0.1 ± 0.4 (0-1.1)	-	-	-	-	-	-	-	-	-
m/z: 59,94,79,43,105	1174	0.9 ± 0.6 (0-1.6)	0.7 ± 0.7 (0-1.6)	0.5 ± 0.5 (0-1.3)	0.7 ± 0.5 (0-1.5)	-	-	-	-	-	-	-	-
m/z: 95,150,41,79,55	1183	0.3 ± 0.3 (0-1)	0.3 ± 0.4 (0-1)	0.3 ± 0.3 (0-0.8)	0.2 ± 0.2 (0-0.4)	-	-	-	-	-	-	-	-

m/z: 82,67,71,43,53	1198	0.3 ± 0.8 (0-2.1)	-	0.2 ± 0.6 (0-1.5)	-	-	-	-	-	-	-	-	-
m/z: 43,109,81,67,152	1208	-	-	-	-	-	-	-	-	0.1 ± 0.2 (0-0.4)	-	tr	-
m/z: 43,109,81,152,67	1209	1.3 ± 1.5 (0-3.4)	2.1 ± 1.3 (0-3.4)	1 ± 1.1 (0-2.5)	1.7 ± 1.2 (0-3.1)	-	-	-	-	-	-	-	-
m/z: 43,79,94,59,105	1231	-	-	-	-	0.3 ± 0.6 (0-1.2)	-	tr	-	-	-	-	-
m/z: 150,107,91,39,79	1233	tr	-	-	0.1 ± 0.2 (0-0.5)	-	-	-	-	-	-	-	-
m/z: 71,43,98,83,55	1240	-	-	-	-	-	-	-	-	0.2 ± 0.3 (0-0.6)	-	tr	0.2 ± 0.3 (0-0.7)
m/z: 125,83,70,55,43	1246	-	-	-	-	-	tr	-	tr	-	-	-	-
m/z: 95,67,41,82,39	1253	tr	-	-	-	-	-	-	-	-	-	-	-
m/z: 69,41,79,95,190	1279	-	-	-	-	0.3 ± 0.7 (0-1.4)	-	tr	-	-	-	-	-
m/z: 104,43,57,77,68	1311	-	-	-	-	-	tr	-	0.1 ± 0.2 (0-0.3)	-	-	-	-
m/z: 93,41,55,119,70	1366	-	-	-	-	-	-	-	-	tr	tr	tr	tr
m/z: 161,189,84,71,55	1386	-	-	-	-	-	tr	-	tr	-	-	-	-
m/z: 57,43,71,150,91	1399	0.1 ± 0.4 (0-1)	tr	0.3 ± 0.5 (0-1.2)	-	-	-	-	-	-	-	-	-
m/z: 150,107,91,135,79	1428	1 ± 0.7 (0-1.9)	0.7 ± 0.7 (0-1.7)	0.8 ± 0.9 (0-2.2)	0.2 ± 0.4 (0-0.8)	-	-	-	-	-	-	-	-
m/z: 79,108,39,82,95	1434	0.1 ± 0.2 (0-0.5)	-	-	0.2 ± 0.3 (0-0.8)	-	-	-	-	-	-	-	-
m/z: 119,93,41,69,107	1449	-	-	-	-	-	-	-	-	0.2 ± 0.1 (0-0.4)	0.6 ± 0.5 (0-1.2)	0.2 ± 0.3 (0-0.7)	0.4 ± 0.3 (0-0.9)
m/z: 105,41,57,77,91	1484	-	-	-	-	tr	-	tr	-	-	-	-	-
m/z: 43,105,91,55,132	1496	tr	-	tr	-	-	-	-	-	-	-	-	-
m/z: 104,57,85,41,77	1499	-	-	-	-	-	-	-	-	0.1 ± 0.2 (0-0.5)	1.4 ± 2.8 (0-5.7)	-	tr
m/z: 161,57,91,105,119	1541	-	-	-	tr	-	-	-	-	-	-	-	-
m/z: 69,162,41,81,131	1586	-	-	-	-	-	tr	-	tr	-	-	-	-

Butterfly-pollinated plant species	**RI**	***L. alba* +**				***L. camara* +**							

		Control		Fed		Control		Fed	
		T1	**T2**	**T1**	**T2**	**T1**	**T2**	**T1**	**T2**
Total number of compounds		44	41	44	41	4	8	6	7
Nsamples (Nplants)		3 (3)	2 (2)	3 (3)	2 (2)	2 (2)	2 (2)	2 (2)	2 (2)
Total amount of scent emitted (ng/ flower/ h)		21.0 ± 2.8	79.6 ± 85.1	14.5 ± 1.1	85.1 ± 102.6	160.4 ± 70.3	173.9 ± 89.5	286.0 ± 260.6	127.1 ± 64.2
Alifatic compounds									
4-Methyl-3-pentene-2-ol*	793	tr	0.1 ± 0.2 (0-0.2)	0.1 ± 0.2 (0-0.3)	0.8 ± 1.2 (0-1.7)	-	-	-	-
Hexanal*	796	-	0.1 ± 0.2 (0-0.3)	-	0.1 ± 0.1 (0-0.2)	-	-	-	-
α-2-Methyl-2-vinyl-5-hydroxytetrahydrofuran	928	0.5 ± 0.3 (0.2-0.9)	1.2 ± 0.6 (0.7-1.6)	0.7 ± 0.7 (0.07-1.4)	1.8 ± 1.4 (0.8-2.8)	-	-	-	-
2-Methyl-2-vinyl-5-hydroxytetrahydrofuran	946	0.4 ± 0.3 (0.1-0.8)	0.7 ± 0.6 (0.3-1.1)	0.6 ± 0.6 (0.04-1.3)	0.9 ± 0.5 (0.5-1.3)	-	-	-	-
3-Octanol*	993	0.1 ± 0.1 (0.05-0.2)	0.2 ± 0.007 (0.22-0.23)	0.1 ± 0.07 (0.07-0.2)	0.3 ± 0.2 (0.1-0.5)	-	-	-	-
Methyl octanoate*	1120	-	-	-	-	43 ± 20.1 (28.7-57.2)	1.3 ± 1.8 (0-2.6)	33.2 ± 46.9 (0-66.4)	-
(Z)-3-Hexenyl butyrate*	1188	0.1 ± 0.2 (0.03-0.4)	tr	0.1 ± 0.1 (0.04-0.3)	0.3 ± 0.5 (0-0.7)	-	-	-	-
Methyl decanoate	1322	-	-	-	-	-	1.8 ± 2.5 (0-3.6)	-	2.8 ± 4.0 (0-5.7)
Aromatic compounds									
Benzaldehyde*	963	0.3 ± 0.1 (0.1-0.4)	0.4 ± 0.1 (0.3-0.5)	0.2 ± 0.2 (0.06-0.4)	0.7 ± 0.6 (0.3-1.2)	-	5.4 ± 7.6 (0-10.8)	15.4 ± 21.9 (0-30.9)	3.3 ± 4.7 (0-6.7)
Methyl salicylate	1204	1 ± 1.3 (0.2-2.6)	0.8 ± 0.6 (0.3-1.3)	0.7 ± 0.8 (0.1-1.6)	0.9 ± 0.1 (0.8-1)	-	7.6 ± 10.8 (0-15.2)	-	11.8 ± 16.8 (0-23.7)
Terpenoids									
6-Methyl-5-hepten-2-one*	987	2 ± 0.7 (1.2-2.7)	3 ± 0.6 (2.5-3.4)	1.7 ± 0.9 (0.6-2.3)	2.3 ± 3.3 (0-4.6)	-	5.2 ± 7.4 (0-10.4)	16.1 ± 22.7 (0-32.2)	3.7 ± 5.3 (0-7.5)
β-Myrcene*	990	0.9 ± 0.2 (0.7-1.2)	0.7 ± 1 (0-1.5)	1 ± 0.1 (0.9-1.2)	0.8 ± 1.1 (0-1.6)	-	-	-	-
Limonene*	1036	0.2 ± 0.3 (0-0.6)	0.2 ± 0.4 (0-0.5)	0.1 ± 0.1 (0-0.3)	0.4 ± 0.5 (0-0.8)	-	-	-	-

Lavender lactone*	1044	0.6 ± 0.2 (0.4-1)	1.4 ± 0.6 (0.9-1.9)	0.8 ± 0.7 (0.06-1.5)	1.4 ± 0.5 (1-1.8)	-	-	-	-
(E)-β-Ocimene*	1049	0.4 ± 0.1 (0.3-0.6)	0.7 ± 0.1 (0.6-0.8)	0.9 ± 0.5 (0.4-1.4)	0.7 ± 0.1 (0.5-0.8)	-	20.4 ± 14.6 (10.0-30.7)	11.7 ± 16.5 (0-23.4)	5.0 ± 7.1 (0-10.1)
(E)-Linalool oxide (furanoid)*	1092	0.5 ± 0.1 (0.3-0.6)	0.5 ± 0.06 (0.5-0.6)	0.5 ± 0.1 (0.3-0.7)	0.7 ± 0.1 (0.6-0.8)	-	-	-	-
Linalool*	1099	58.5 ± 27.7 (26.6-76.7)	75.6 ± 1.5 (74.6-76.7)	68.9 ± 2.5 (66.6-71.6)	69.0 ± 5.9 (64.8-73.3)	33.5 ± 47.5 (0-67.1)	54.9 ± 19.3 (41.2-68.5)	6.6 ± 9.4 (0-13.3)	66.9 ± 4.0 (64.1-69.8)
Hotrienol	1104	3.4 ± 0.8 (2.4-4)	2.0 ± 2.9 (0-4.1)	2.5 ± 2.4 (0-4.8)	6.0 ± 0.7 (5.5-6.5)	-	-	-	-
(E)-4,8-dimethyl-1,3,7-nonatriene*	1119	1.1 ± 0.6 (0.4-1.6)	0.9 ± 0.07 (0.9-1.0)	0.9 ± 0.3 (0.5-1.2)	1.7 ± 0.3 (1.4-2.0)	2.0 ± 2.8 (0-4.0)	3.1 ± 4.5 (0-6.3)	-	6.0 ± 8.5 (0-12.0)
Ocimene derivative	1125	0.3 ± 0.08 (0.2-0.4)	0.3 ± 0.1 (0.2-0.4)	0.3 ± 0.07 (0.2-0.4)	0.4 ± 0.1 (0.3-0.5)	-	-	-	-
Allo-Ocimene*	1130	-	-	-	-	-	-	-	-
para-1,3,8-Menthatriene	1134	0.4 ± 0.7 (0-1.3)	0.4 ± 0.6 (0-0.9)	0.8 ± 0.7 (0-1.5)	0.5 ± 0.8 (0-1.1)	-	-	-	-
(E)-linalool oxide (pyranoid)*	1175	0.1 ± 0.04 (0.06-0.1)	tr	0.1 ± 0.03 (0.08-0.1)	tr	-	-	-	-
α-Terpineol	1198	0.4 ± 0.1 (0.2-0.5)	0.7 ± 0.1 (0.6-0.9)	0.5 ± 0.1 (0.4-0.7)	0.9 ± 0.6 (0.4-1.3)	-	-	-	-
Neral	1245	tr	tr	0.1 ± 0.08 (0-0.2)	0.1 ± 0.1 (0-0.2)	-	-	-	-
Geranial*	1273	0.7 ± 0.2 (0.5-1)	0.3 ± 0.4 (0-0.6)	0.7 ± 0.3 (0.3-1)	0.3 ± 0.4 (0-0.6)	-	-	-	-
β-Bourbonene*	1407	3.1 ± 2.6 (1.5-6.2)	1 ± 0.1 (0.9-1.1)	2.5 ± 1.5 (0.7-3.6)	0.8 ± 1.1 (0.08-1.6)	-	-	-	-
β-Caryophyllene*	1444	2.9 ± 2.7 (0.6-6.0)	0.8 ± 0.4 (0.4-1.1)	3.3 ± 2.4 (0.6-5.2)	0.2 ± 0.2 (0.06-0.4)	-	-	-	-
(E)-β-Farnesene*	1461	4.0 ± 4.6 (1.0-9.3)	-	1.3 ± 1.4 (0-2.8)	-	-	-	-	-
Germacrene D*	1497	0.3 ± 0.1 (0.2-0.4)	tr	0.8 ± 0.4 (0.5-1.3)	0.3 ± 0.5 (0-0.7)	-	-	-	-
α-Amorphene	1520	0.6 ± 0.8 (0.001-1.6)	0.2 ± 0.3 (0-0.5)	0.5 ± 0.07 (0.4-0.6)	0.1 ± 0.2 (0-0.3)	-	-	-	-
Kessane	1705	3.3 ± 3.7 (1.1-7.7)	2.1 ± 1.6 (0.9-3.3)	1.7 ± 1.6 (0-3.1)	1.5 ± 2.1 (0-3.0)	-	-	-	-
Unidentified compounds									
m/z: 133,151,43,95,67	888	tr	-	tr	-	-	-	-	-

m/z: 81,41,69,79,150	1015	0.3 ± 0.2 (0.1-0.6)	0.4 ± 0.1 (0.2-0.5)	0.3 ± 0.1 (0.1-0.4)	0.3 ± 0.4 (0-0.6)	-	-	-	-
m/z: 91,119,134,41,77	1082	0.1 ± 0.09 (0.04-0.2)	0.1 ± 0.02 (0.1-0.2)	0.2 ± 0.1 (0.1-0.3)	0.1 ± 0.02 (0.1-0.1)	-	-	-	-
m/z: 59,94,79,43,105	1174	0.4 ± 0.3 (0.1-0.8)	0.4 ± 0.6 (0-0.9)	1.0 ± 0.3 (0.7-1.3)	0.2 ± 0.3 (0-0.4)	-	-	-	-
m/z: 43,135,107,91,65	1203	-	-	-	-	21.3 ± 30.2 (0-42.7)	-	16.7 ± 23.7 (0-33.5)	-
m/z: 43,109,81,67,152	1208	0.4 ± 0.2 (0.1-0.6)	0.4 ± 0.1 (0.4-0.5)	0.6 ± 0.2 (0.3-0.9)	1.3 ± 0.8 (0.7-1.9)	-	-	-	-
m/z: 43,94,68,55,79	1290	tr	tr	tr	0.1 ± 0.1 (0-0.2)	-	-	-	-
m/z: 82,73,43,67,71	1322	tr	-	tr	-	-	-	-	-
m/z: 82,43,71,67,55	1332	1.6 ± 2.1 (0.3-4.1)	0.4 ± 0.6 (0-0.9)	0.5 ± 0.5 (0.06-1.2)	0.1 ± 0.2 (0-0.3)	-	-	-	-
m/z: 93,55,41,85,77	1381	1.3 ± 1.8 (0.2-3.5)	0.2 ± 0.03 (0.2-0.3)	0.3 ± 0.1 (0.2-0.5)	0.4 ± 0.2 (0.2-0.6)	-	-	-	-
m/z: 161,120,105,91,43	1441	0.4 ± 0.3 (0.2-0.7)	0.2 ± 0.04 (0.1-0.2)	0.5 ± 0.4 (0.2-1.0)	0.2 ± 0 (0.2-0.2)	-	-	-	-
m/z: 93,121,105,41,55	1448	0.1 ± 0.3 (0-0.5)	tr	0.2 ± 0.3 (0-0.6)	tr	-	-	-	-
m/z: 119,93,41,69,107	1449	0.3 ± 0.3 (0-0.7)	tr	0.2 ± 0.2 (0-0.5)	tr	-	-	-	-
m/z: 161,57,91,105,119	1541	0.1 ± 0.1 (0-0.2)	-	0.3 ± 0.2 (0-0.5)	-	-	-	-	-
m/z: 43,91,121,79,107	1739	4.7 ± 7.4 (0-13.3)	1.4 ± 1.1 (0.5-2.2)	1.1 ± 0.9 (0-1.7)	1.1 ± 1.6 (0-2.3)	-	-	-	-
m/z: 43,93,55,107,85	1767	0.5 ± 0.9 (0-1.6)	tr	tr	tr	-	-	-	-
m/z: 43,95,55,81,159	1779	1.3 ± 1.9 (0.1-3.5)	0.2 ± 0.1 (0.1-0.3)	0.2 ± 0.1 (0.1-0.4)	0.2 ± 0.2 (0.1-0.4)	-	-	-	-

Hawkmoth-pollinated plant species	**RI**	***T. formosa***			
		Control		**Fed**	
		T1	**T2**	**T1**	**T2**
Total number of compounds		27	29	27	29
Nsamples (Nplants)		4 (4)	4 (4)	4 (4)	4 (4)

Total amount of scent emitted (ng/ flower/ h)		646.8 ± 148.4	437.9 ± 108.5	664.4 ± 81.3	240.8 ± 110.5
Alifatic compounds					
Hexanal*	796	-	tr	-	tr
1-Hexanol*	864	tr	tr	tr	tr
1-Octen-3-ol*	976	tr	tr	tr	tr
Aromatic compounds					
Benzaldehyde*	963	0.2 ± 0.1 (0-0.4)	0.2 ± 0.1 (0.06-0.3)	0.2 ± 0.1 (0-0.4)	0.2 ± 0.1 (0.05-0.5)
Benzyl alcohol*	1036	14.0 ± 9.0 (0.5-18.7)	9.8 ± 5.5 (2.1-15.3)	13.1 ± 8.8 (0.3-20.0)	8.6 ± 7.0 (1.1-18.1)
Methyl benzoate*	1100	43.5 ± 6 (37.9-51.9)	10.2 ± 3.1 (6.5-13.8)	45.9 ± 4.5 (42.0-52.4)	15.1 ± 2.7 (11-16.8)
2-Phenylethanol*	1117	tr	0.1 ± 0.04 (0.08-0.1)	tr	0.1 ± 0.05 (0.06-0.1)
para-Vinylanisole*	1159	-	tr	-	0.1 ± 0.1 (0-0.2)
Benzyl acetate	1167	tr	tr	tr	tr
Methyl salicylate	1204	0.5 ± 1.0 (0.003-2.1)	0.8 ± 1.6 (0-3.4)	0.6 ± 1.3 (0.02-2.6)	0.4 ± 0.7 (0-1.6)
para-Anisaldehyde*	1263	-	tr	-	tr
Miscelaneous ciclic compounds					
2,2,6-Trimethy-6-vinyldihydro-2H-pyran-3(4H)-one*	1112	0.8 ± 0.08 (0.7-0.9)	0.4 ± 0.1 (0.2-0.6)	0.9 ± 0.07 (0.8-0.9)	0.4 ± 0.2 (0.2-0.7)
N-bearing compounds					
2-Methylbutanenitrile	716	0.1 ± 0.2 (0-0.4)	0.2 ± 0.1 (0-0.4)	tr	0.3 ± 0.3 (0-0.9)
Isobutylaldoxime	747	-	tr	-	tr
syn-3-Methylbutylaldoxime*	848	0.3 ± 0.1 (0.2-0.6)	0.3 ± 0.04 (0.2-0.3)	0.3 ± 0.06 (0.2-0.4)	0.4 ± 0.1 (0.3-0.5)
syn-2-Methylbutylaldoxime	851	1.9 ± 2.3 (0.7-5.4)	1.7 ± 1.2 (1-3.5)	1.7 ± 1.9 (0.5-4.6)	3.0 ± 3.0 (1.4-7.6)
anti-2-Methylbutylaldoxime	858	0.8 ± 0.9 (0.2-2.3)	0.7 ± 0.3 (0.5-1.2)	0.8 ± 0.8 (0.1-2)	1.2 ± 1.0 (0.6-2.7)

Methylnicotinate*	1143	0.2 ± 0.08 (0.1-0.3)	0.8 ± 0.2 (0.6-1.1)	0.2 ± 0.06 (0.1-0.3)	0.6 ± 0.2 (0.4-0.9)
Terpenoids					
6-Methyl-5-hepten-2-one*	987	tr	0.1 ± 0.03 (0.06-0.1)	tr	0.2 ± 0.07 (0.1-0.3)
β-Myrcene*	990	tr	-	tr	-
(Z)-β-Ocimene*	1037	tr	-	tr	-
(E)-β-Ocimene*	1049	0.1 ± 0.08 (0-0.2)	tr	0.1 ± 0.1 (0-0.2)	0.1 ± 0.1 (0.001-0.3)
(Z)-Linalool oxide (furanoid)*	1076	2.9 ± 0.5 (2.3-3.6)	2.1 ± 0.7 (1.2-3.0)	2.9 ± 0.1 (2.6-3.1)	2.7 ± 1.1 (1.6-4.3)
(E)-Linalool oxide (furanoid)*	1092	0.6 ± 0.1 (0.5-0.8)	0.5 ± 0.1 (0.3-0.6)	0.6 ± 0.04 (0.5-0.6)	0.5 ± 0.2 (0.3-0.8)
Allo-Ocimene*	1130	tr	tr	tr	tr
(Z)-linalool oxide (pyranoid)	1175	2.9 ± 1.3 (1.3-4.1)	1.2 ± 1.3 (0.09-3.1)	3.5 ± 1.3 (1.6-4.9)	0.9 ± 0.5 (0.3-1.3)
(E)-linalool oxide (pyranoid)*	1175	29.8 ± 2.5 (26.7-32.8)	69.8 ± 4.6 (65.3-74.2)	28.1 ± 2.4 (25.5-30.3)	63.9 ± 7.8 (52.4-69.7)
(3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	1584	tr	tr	tr	tr
Benzyl benzoate*	1785	0.1 ± 0.2 (0-0.6)	tr	0.1 ± 0.2 (0-0.4)	tr
Unidentified compounds					
m/z: 81,43,109,71,55	946	tr	-	tr	-
m/z: 68,43,94,59,85	1237	tr	tr	tr	tr
m/z: 43,94,68,55,79	1290	-	tr	-	tr

Hummingbird-pollinated plant species	**RI**	***Z. montana***			
		Control		**Fed**	
		T1	**T2**	**T1**	**T2**
Total number of compounds		7	16	20	13
Nsamples (Nplants)		2 (2)	4 (4)	5 (5)	6 (6)
Total amount of scent emitted (ng/ flower/ h)		27.5 ± 30.6	87.1 ± 128.7	53.7 ± 105.5	68.8 ± 125.6

Alifatic compounds					
Ethyl 2-methylacrylate	783	7.4 ± 10.4 (0-14.8)	-	1.5 ± 3.3 (0-7.5)	27.9 ± 44.4 (0-100)
1-Hexanol*	864	0.7 ± 1.0 (0-1.4)	4.1 ± 8.2 (0-16.5)	0.2 ± 0.5 (0-1.3)	-
2-Methylbutyl acetate*	874	0.3 ± 0.5 (0-0.7)	1.2 ± 1.6 (0-3.5)	0.2 ± 0.6 (0-1.4)	-
1-Octen-3-ol*	976	-	0.3 ± 0.7 (0-1.5)	0.2 ± 0.6 (0-1.4)	-
3-Octanone	987	-	-	0.7 ± 1.5 (0-3.5)	0.2 ± 0.6 (0-1.7)
2-Ethylhexoic acid	1111	-	-	12.7 ± 28.4 (0-63.5)	8.9 ± 21.9 (0-53.8)
Tridecane*	1300	-	10.4 ± 17.6 (0-36.7)	-	-
Tetradecane	1401	-	8.5 ± 13.7 (0-28.8)	0.6 ± 1.4 (0-3.3)	0.8 ± 2.1 (0-5.1)
Aromatic compounds					
Benzaldehyde*	963	31.8 ± 45.0 (0-63.6)	5.0 ± 10.0 (0-20.0)	9.0 ± 20.2 (0-45.3)	-
Benzyl alcohol*	1036	44.4 ± 35.6 (19.2-69.7)	40.3 ± 28.8 (11.0-79.8)	14.2 ± 19.9 (0-41.5)	12.9 ± 23.1 (0-57.0)
Methyl benzoate*	1100	-	-	15.0 ± 33.5 (0-75.0)	2.0 ± 4.9 (0-12.0)
Benzyl acetate	1167	-	0.2 ± 0.4 (0-0.8)	19.9 ± 44.6 (0-99.8)	tr
para-Acetylacetophenone	1464	-	-	6.6 ± 14.8 (0-33.1)	6.8 ± 16.7 (0-41.0)
Terpenoids					
Lavender lactone*	1044	-	1.0 ± 2.0 (0-4.0)	0.9 ± 2.1 (0-4.8)	-
(Z)-Linalool oxide (furanoid)*	1076	9.8 ± 13.8 (0-19.6)	4.7 ± 7.4 (0-15.7)	0.7 ± 1.7 (0-3.9)	4.7 ± 11.5 (0-28.3)
(E)-Linalool oxide (furanoid)*	1092	-	0.7 ± 1.4 (0-2.8)	0.4 ± 0.9 (0-2.2)	-
Linalool*	1099	-	-	4.9 ± 11.1 (0-24.9)	10.2 ± 17.2 (0-41.1)
(E)-4,8-dimethyl-1,3,7-nonatriene*	1119	-	-	0.02 ± 0.05 (0-0.1)	16.6 ± 40.7 (0-99.8)

Linalyl acetate	1256	-	3.2 ± 6.4 (0-12.8)	0.5 ± 1.2 (0-2.9)	-
Geranyl acetate*	1384	-	2.0 ± 2.5 (0-5.3)	-	-
α-Copaene*	1395	-	2.8 ± 4.1 (0-8.8)	-	-
β-Caryophyllene*	1444	-	-	9.6 ± 21.5 (0-48.1)	0.9 ± 2.3 (0-5.6)
1-nor-Bourbonanone	1588	-	2.3 ± 4.6 (0-9.2)	-	-
Unidentified compounds					
m/z: 57,71,43,85,127	1056	5.3 ± 7.5 (0-10.6)	12.7 ± 22.4 (0-46.2)	1.2 ± 2.8 (0-6.3)	7.5 ± 18.4 (0-45)

Appendix 2. Detailed statistics of the PERMANOVAs (9999 permutations) to evaluate if florivory affected the total amount of scent emitted by flowers, the qualitative composition of floral scent, and the relative composition of floral scent in eight savanna plant species (effect of 'Treatment*Time'), considering plant individual as a random factor. Statistically significant results are marked in bold.

Amphilophium mansoanum

Total amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	3,23E+13	3,23E+13	0,13283	0,7224
Time	1	1,55E+15	1,55E+15	3,8327	0,1234
Plant	6	3,81E+15	6,36E+14	3,1394	0,1889
Treatment*Time	1	4,83E+13	4,83E+13	0,2387	0,6553
Treatment*Plant	5	1,26E+15	2,51E+14	1,2404	0,4562
Time*Plant	4	1,69E+15	4,23E+14	2,0869	0,2834
Residuals	3	6,08E+14	2,03E+14		
Total	21	8,34E+15			
Relative amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	511,18	511,18	1,0993	0,3954
Time	1	2696,2	2696,2	3,133	0,0872
Plant	6	4972	828,66	1,3333	0,3101
Treatment*Time	1	657,02	657,02	1,0571	0,4006
Treatment*Plant	5	2168,4	433,69	0,6978	0,7172
Time*Plant	4	3531,9	882,98	1,4207	0,3606
Residuals	3	1864,5	621,51		
Total	21	15533			

Byrsonima intermedia

Total amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	7,71E+15	7,71E+15	6,6456	0,089
Time	1	9,43E+14	9,43E+14	0,49823	0,6518
Plant	4	3,73E+16	9,32E+15	21,982	0,1616
Treatment*Time	1	1,90E+15	1,90E+15	4,471	0,2661
Treatment*Plant	3	4,21E+15	1,40E+15	3,3137	0,3821
Time*Plant	2	4,15E+15	2,08E+15	4,8971	0,2931
Residuals	1	4,24E+14	4,24E+14		
Total	13	5,20E+16			
Relative amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	383,52	383,52	0,32623	0,7699
Time	1	2564,6	2564,6	1,6522	0,3121
Plant	4	6755,7	1688,9	1,5386	0,4978
Treatment*Time	1	1120,1	1120,1	1,0204	0,3528
Treatment*Plant	3	3604,7	1201,6	1,0947	0,5621
Time*Plant	2	3218,1	1609	1,4659	0,436
Residuals	1	1097,7	1097,7		
Total	13	21646			

Centrosema pubescens

Total amount	Df	Sum of squares	Mean squares	Pseudo-F	p

Treatment	1	7,34E+12	7,34E+12	8,42E-02	0,7772
Time	1	3,65E+13	3,65E+13	1,98E-02	0,9161
Plant	3	3,90E+15	1,30E+15	4,3655	0,1337
Treatment*Time	1	1,82E+14	1,82E+14	0,61264	0,4909
Treatment*Plant	3	2,61E+14	8,71E+13	0,29292	0,8249
Time*Plant	3	5,53E+15	1,84E+15	6,1997	0,0838
Residuals	3	8,92E+14	2,97E+14		
Total	15	1,08E+16			
Relative amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	478,15	478,15	0,71209	0,4639
Time	1	4031,7	4031,7	4,9876	**0,0482**
Plant	3	4955,9	1652	3,6875	0,0633
Treatment*Time	1	70,447	70,447	0,15725	0,8405
Treatment*Plant	3	2014,4	671,47	1,4989	0,3561
Time*Plant	3	2425	808,35	1,8044	0,2432
Residuals	3	1344	447,99		
Total	15	15320			
Lantana camara					
Total amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	2,04E+10	2,04E+10	1,08E-02	0,5042
Time	1	3,34E+14	3,34E+14	0,96379	0,4891
Plant	1	3,59E+14	3,59E+14	78,385	0,1143
Treatment*Time	1	2,90E+12	2,90E+12	0,63291	0,5313
Treatment*Plant	1	1,90E+12	1,90E+12	0,41401	0,5439
Time*Plant	1	3,46E+14	3,46E+14	75,608	0,1241
Residuals	1	4,58E+12	4,58E+12		
Total	7	1,05E+15			
Relative amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	329,53	329,53	0,19199	0,5014
Time	1	7143,5	7143,5	2,7339	0,2567
Plant	1	4409,9	4409,9	7,0906	0,1161
Treatment*Time	1	1837,3	1837,3	2,9541	0,1557
Treatment*Plant	1	1716,4	1716,4	2,7598	0,1547
Time*Plant	1	2612,9	2612,9	4,2012	0,1611
Residuals	1	621,94	621,94		
Total	7	18671			
Lippia alba					
Total amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	1,88E+15	1,88E+15	0,90105	0,5068
Time	1	3,98E+13	3,98E+13	0,151	0,6444
Plant	2	8,36E+15	4,18E+15	3,557	0,3556
Treatment*Time	1	9,73E+13	9,73E+13	8,28E-02	0,8166
Treatment*Plant	2	4,41E+15	2,21E+15	1,8772	0,4439
Time*Plant	1	2,64E+14	2,64E+14	0,22436	0,7145
Residuals	1	1,17E+15	1,17E+15		
Total	9	1,77E+16			
Relative amount	Df	Sum of squares	Mean squares	Pseudo-F	p

Treatment	1	93,236	93,236	0,25412	0,7927
Time	1	665,03	665,03	1,5435	0,3203
Plant	2	938,86	469,43	0,7846	0,6209
Treatment*Time	1	307,7	307,7	0,51429	0,6463
Treatment*Plant	2	675,94	337,97	0,56488	0,7118
Time*Plant	1	430,86	430,86	0,72013	0,5942
Residuals	1	598,3	598,3		
Total	9	3495,7			
Tocoyena formosa					
Total amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	1,81E+14	1,81E+14	1,5659	0,3539
Time	1	2,25E+15	2,25E+15	25,976	**0,0305**
Plant	3	2,08E+14	6,95E+13	2,8956	0,2112
Treatment*Time	1	2,59E+14	2,59E+14	10,8	**0,0493**
Treatment*Plant	3	3,47E+14	1,16E+14	4,8224	0,1187
Time*Plant	3	2,60E+14	8,66E+13	3,6089	0,1648
Residuals	3	7,20E+13	2,40E+13		
Total	15	3,58E+15			
Relative amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	53,723	53,723	2,169	0,2226
Time	1	7307	7307	Negative	
Plant	3	1866,9	622,31	17,692	**0,0157**
Treatment*Time	1	72,956	72,956	2,0741	0,1849
Treatment*Plant	3	74,306	24,769	0,70417	0,6483
Time*Plant	3	-589,54	-196,51	Negative	
Residuals	3	105,52	35,174		
Total	15	8890,9			
Zeyheria montana					
Total amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	1,32E+14	1,32E+14	5,78E-02	0,886
Time	1	1,03E+12	1,03E+12	1,22E-02	0,912
Plant	5	6,47E+15	1,29E+15	6,6621	0,2941
Treatment*Time	1	1,60E+14	1,60E+14	0,82445	0,705
Treatment*Plant	3	8,92E+15	2,97E+15	15,301	0,175
Time*Plant	4	8,86E+13	2,21E+13	0,11402	0,9618
Residuals	1	1,94E+14	1,94E+14		
Total	16	1,75E+16			
Relative amount	Df	Sum of squares	Mean squares	Pseudo-F	p
Treatment	1	3032,7	3032,7	1,5539	0,2578
Time	1	1382,4	1382,4	0,66235	0,6881
Plant	5	28214	5642,9	9,4496	**0,0091**
Treatment*Time	1	687,44	687,44	1,1512	0,3053
Treatment*Plant	3	7209,7	2403,2	4,0245	0,1135
Time*Plant	4	11701	2925,3	4,8987	0,0601
Residuals	1	597,15	597,15		
Total	16	63457			

Appendix 3. Pairwise comparisons of the effect of 'Treatment*Time' for the total amount of scent produced by *Tocoyena formosa* flowers. Statistically significant results are marked in bold.

Groups	**t**	**p**
T1 Control - T1 Fed	0,1736	0,8545
T1 Control - T2 Control	2,5949	0,0862
T1 Control - T2 Fed	3,5163	0,0673
T1 Fed - T2 Control	3,3183	0,0681
T1 Fed - T2 Fed	7,3153	**0,0305**
T2 Control - T2 Fed	4,2366	**0,0473**

CAPÍTULO 5

INTERSECÇÃO ENTRE FLORIVORIA E POLINIZAÇÃO: ALTERAÇÕES NO CONTORNO FLORAL NÃO DESENCORAJAM A POLINIZAÇÃO POR BEIJA-FLORES

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Florivory and Pollination Intersection: Changes in Floral Trait Expression Do Not Discourage Hummingbird Pollination

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Many flowers are fed on by florivores, but we know little about if and how feeding on flowers affects their visual and chemical advertisement and nectar resource, which could disrupt pollination. Here, we investigated if damages caused by florivores compromise a Neotropical hummingbird pollination system, by modifying the floral advertisements and the nectar resource. We surveyed natural florivory levels and patterns, examined short-term local effects of floral damages caused by the most common florivore, a caterpillar, on floral outline, intra-floral color pattern and floral scent, as well as on the amount of nectar. Following, we experimentally tested if the most severe florivory pattern affected hummingbird pollination. The feeding activity of the most common florivore did not alter the intra-floral color pattern, floral scent, and nectar volume, but changed the corolla outline. However, this change did not affect hummingbird pollination. Despite visual floral cues being important for foraging in hummingbirds, our results emphasize that changes in the corolla outline had a neutral effect on pollination, allowing the maintenance of florivore–plant–pollinator systems without detriment to any partner.

Keywords: floral attractants, floral damage, florivory, hummingbird pollination, *Pyrostegia venusta*, floral cues, plant–pollinator communication

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INTRODUCTION

Our knowledge is growing fast concerning the visual and chemical signal diversity involved in plant–pollinator communication (Leonard and Masek, 2014; Schaefer and Ruxton, 2015; Kantsa et al., 2017; Leonard and Francis, 2017). However, we know little about if and how florivores, by feeding on flowers, affect these signals and consequently the visual (Johnson et al., 1995; Krupnick et al., 1999) and chemical advertisements (McCall and Irwin, 2006; Lucas-Barbosa et al., 2011; Späthe, 2013; Vega-Polanco et al., 2020), which could disrupt the communication between flowers and pollinators. Indeed, there is accumulated evidence that pollinators do respond to damages caused by florivores, for example, performing less visits (Krupnick et al., 1999; Mothershead and Marquis, 2000; Pohl et al., 2006; McCall, 2008; Tsuji et al., 2016; Muola et al., 2017), which has negative effects on pollination success (Kessler and Halitschke, 2009; Haas and Lortie, 2020).

The effects of florivore feeding can be local, restricted to a single flower, and/or systemic, involving the activation of phytohormonal signaling pathways (Chrétien et al., 2018; Rusman et al., 2019a). So far, most studies have focused on the outcome of florivory on plant fitness (Haas and Lortie, 2020; Boaventura et al., 2022). There is, however, limited knowledge regarding which specific changes in floral advertisement, being them locally or systemically elicited, act as triggers for behavioral responses displayed by pollinators. Indeed, we do not know if florivore-induced changes in a single floral cue are enough to affect pollinator behavior in natural systems or if changes in multiple floral cues are required, as pollinators usually integrate between visual and olfactory cues (Leonard and Masek, 2014; Junker and Parachnowitsch, 2015).

Among such pollinators that use both visual and olfactory cues when looking for flowers are hummingbirds (Altshuler and Nunn, 2001; Hurly and Healy, 2002; Kessler et al., 2008). They are highly specialized pollinators in the Neotropics (Stiles, 1981; Zanata et al., 2017) with a well-developed visual system (Hickman and Robert, 1993; Pritchard et al., 2017; Tyrrell et al., 2018 and references therein). Indeed, there is experimental evidence that hummingbirds recognize specific floral shapes and prefer visiting those associated to their bill shape and size (Maglianesi et al., 2015; Rico-Guevara et al., 2021). As florivory implies damage to the flowers, which affects flower integrity and shape, we expect that these changes *per se* could be enough to interfere in the visual communication between flowers and hummingbirds, causing hummingbirds to neglect damaged flowers. Even though there was a long-held belief that olfaction is not involved in the location and selection of flowers by hummingbirds, it is meanwhile known that they can perceive (Steiger et al., 2008; Wester and Lunau, 2017) and respond (Kessler et al., 2008) to floral volatile compounds. By feeding on floral tissue that is potentially involved in the biosynthesis and emission of floral scent, florivores might affect the total amount of scent emitted by flowers (Zangerl and Berenbaum, 2009; Tunes and Guimarães, 2020) and the chemical composition of floral scent (Lucas-Barbosa et al., 2013; Farré-Armengol et al., 2015). Moreover, these florivory-induced changes in floral scent can be expressed exclusively locally, on the damaged flower itself, or systemically, leading to changes in the damaged flower as well as in undamaged flowers of the same plant (Dicke et al., 1990; Turlings and Tumlinson, 1992; Potting et al., 1995; Röse et al., 1996; Lucas-Barbosa et al., 2013). In addition to the advertisement, florivory might also have effects on floral resources. Although it is not expected that florivores deplete the nectar resource used by hummingbirds, since they often feed on floral tissues other than nectaries and not on nectar itself (Xiao et al., 2021), the injuries caused by florivores could interfere with nectar secretion by altering plant physiological pathways. In fact, by feeding on plant tissues, herbivores can alter jasmonate pathways, which are involved in both plant defense (Xie et al., 1998; Chrétien et al., 2018) and nectar secretion (Radhika et al., 2010). Therefore, we considered that florivores could act indirectly on floral nectar resources, beyond merely acting on floral advertisements. Thus, as flowers are the pollination units that are recognized and pursued by pollinators, direct and indirect changes that happen during a flower lifetime might have significant consequences for the pollination process. In this study, we focused on short-term local effects of florivory on floral advertisement, floral resource, and pollination. Specifically, we aimed to elucidate if florivory affects the visual and olfactory advertisement, the nectar resource, and finally the pollination success in a hummingbird-pollinated plant. Therefore, we investigated (i) natural florivory levels and florivores (ii) if and which florivory-induced local changes occur in floral visual and olfactory advertisements, and (iii) if florivory affects nectar volume. Finally, (iv) we simulated florivory-induced floral changes and experimentally tested if they discourage hummingbird pollination.

MATERIALS AND METHODS

Focal Plant Species, Its Pollinators, and Study Site

Pyrostegia venusta (Ker-Gawl.) Miers (Bignoniaceae) is a neotropical vine (**Figure 1A**) that occurs from the northeast coast of Brazil to the northeast of Argentina (approximately 35°–58° W and 5°–30° S; Pool, 2008). The flowering period of this plant species occurs from April to October, with a peak in July/August (our study period), when there was the highest number of flowers opening per day (Rossatto and Kolb, 2011). This species shows terminal or axillary panicles (**Figure 1B**); zygomorphic flowers with a long tubular orange corolla presenting curved-back lobes; four exerted didynamous stamens; a syncarpous gynoecium with bilocular ovary, long style, and bilabiate stigma (**Figure 1C**) with lobes that close following mechanical stimulation (Pool, 2008; Singh et al., 2009). Flower anthesis starts at approximately 06:30 h and lasts for approximately 48 h (Galetto et al., 1994). *Pyrostegia venusta* is self-compatible (Gobatto-Rodrigues and Stort, 1992), with hermaphroditic and protandrous flowers (Pool, 2008) that show diverse degrees of approach herkogamy. Most flowers present the gynoecium longer than the androecium, whereas others do not (Pool, 2008; Tunes and Guimarães, 2020). We deposited a voucher specimen in the Herbarium BOTU "Irina Delanova de Gemtchujnicov" (voucher number 30788). The authorization for collection of biological samples is registered on Sisgen under #A90A83C.

Floral nectar is sucrose-rich, with a concentration of 28% of sugars (Galetto et al., 1994). It is consumed by hummingbird pollinators, such as *Eupetomena macroura*, *Phaethornis squalidus*, *P. pretrei* (Leone, 2011), *Chlorostilbon lucidus* (previously *C. aureoventris* (Grantsau, 1988)), and *Sappho sparganura* (Galetto et al., 1994). Even though this plant species is self-compatible, and some flowers are capable of autonomous selfing and of being self-pollinated by small bees, hummingbird pollination is crucial for plant reproduction, especially when considering that most of the flowers have approach herkogamy and only hummingbirds are capable of transferring pollen among those flowers (Tunes and Guimarães, 2020).

This study was performed in a natural population growing at the edge of a seasonal tropical forest fragment (at 22°53' S and 48°29' W), in Botucatu municipality, São Paulo state, Brazil. To evaluate the effects of florivory on floral traits and

FIGURE 1 | *Pyrostegia venusta* and its florivores. **(A)** Flowering plant. Scale bar: 20 cm. **(B)** Inflorescence with undamaged open flowers and flower buds. Scale bar: 2.5 cm. **(C)** Recently opened flower. Scale bar: 0.8 cm. **(D)** Natural floral damage with 51–60% of corolla damage. Scale bar: 0.5 cm.

to assess the effect of experimentally simulated floral damages on hummingbird pollination, we randomly assigned different individuals to the investigation of each trait (floral color pattern, floral scent, floral nectar) and for the field experiment, totaling 62 different plants.

Natural Incidence of Florivory and Florivores Associated to It

We randomly designated *P. venusta* plants to assess the percentage of flowers with natural damages to the corolla. We sampled all the first- and second-day flowers from 80% of the plants in the population ($n = 935$ flowers, from 58 plants). Then, we visually estimated the percentage of corolla removal in each damaged flower and classified the flowers into one of the following categories (adapted from Dirzo and Domingues, 1995): 1–3%, 4–5%, 6–10%, 11–20%, 21–30%, 31–40%, 41–50%, and 51–60% of removed tissue. Additionally, we performed 40 h of flower observations in ten different days, during the morning (15 h), afternoon (15 h), and night (10 h), to identify the animals responsible for each type of damage registered on the flowers.

Evaluating the Effects of Natural Florivory on Floral Traits

We evaluated the effect of the most severe damage (category of 51 to 60% of corolla removal) caused by a caterpillar (the most common florivore) on floral outline, intra-floral color pattern and floral scent, as well as on the amount of nectar.

Floral Outline

Floral outline (*sensu* Herrera, 1993) may act as a signal from the perspective of a hummingbird when hovering in front of a flower. Florivores, by feeding on flowers, have the potential to change the floral frontal outline. Here, we investigate if the most common florivore, by feeding on flowers, changes the frontal floral outline, which could lead hummingbirds to neglect damaged flowers.

Intra-floral Color Pattern

When hummingbirds are hovering in front of *P. venusta* flowers, they see the yellowish anthers and stigma against the inner surface of the orange upper petal lobes of the corolla, which act as background (**Figure 1C**). However, when the upper portion of the corolla is removed by florivores, the hummingbirds will see the anthers and stigma against the inner portion of the remaining lower half of the corolla tube (**Figure 1D**). Thus, in damaged flowers, a different flower portion acts as the background for the reproductive structures. Thus, to accurately determine if florivory changes intra-floral color pattern, we associated the human-visible color patterns with UV photography from five intact and five damaged flowers ($n=5$ plants, each containing both types of flowers), which allowed us to see if there was any UV pattern that could be modified by florivory. After stablishing the floral portions/structures responsible for creating the intra-floral color patterns, we measured the spectral reflectance of the anthers, the stigma, the upper petal lobes, and the inner portion of the lower half of the corolla tube (**Supplementary Figure 1**) of 20 flowers ($n=8$ plants). Firstly, we measured the reflectance of the stigma, anthers, and superior corolla lobes of undamaged flowers. Then, we manually caused the damage in the same flowers (the same severe damage caused by the most common florivore and used for the pollination experiments, **Figure 1D**) and measured the reflectance of the inner portion of the lower half of the corolla tube. This is the portion of the corolla that will contrast against the stigma and anthers in the damaged flowers. For UV photography, we used a camera with a modified sensor and lens (Canon EOS Rebel T3i with a 50 mm lens) that only captures UV light from 340 to 400 nm. We illuminated the flowers with a hand-held UV light source, which emits light from 315 to 405 nm, which corresponds to the spectral sensitivity of birds' UV photoreceptors (Herrera et al., 2008; Ödeen and Håstad, 2010). For spectral reflectance, we used a spectrophotometer (Ocean Optics Jaz-EL200 UV–VIS) and collected reflectance data from 300–700 nm. A deuterium–halogen lamp, that emitted light in the range of 215 to 1700 nm, was used as the light source. We used the reflectance of a "diffuse Spectralon reflectance standard" as white standard and the reflectance obtained from inside a black chamber as black standard, according to Lunau et al. (2011). We took all measurements at 45° in relation to the flower floral surface.

To provide a graphical representation of floral color as perceived by hummingbirds, we calculated the color loci of each floral part in the tetrahedron color space model (Vorobyev et al., 1998). We used the D65 standard daylight illumination (Wyszecki and Stiles, 1982) and a standard function of green leaves (AV 400) as the background. To display the contrast as seen by hummingbirds in intact flowers, we calculated the chromatic and achromatic contrasts of the anthers and of the stigma against the upper petal lobes. To display the contrast in damaged flowers, we calculated the chromatic and achromatic contrasts of the anthers and of the stigma against the upper internal portion of the corolla tube. To evaluate if hummingbirds could perceive the contrast between these floral parts, we considered 1.00 just noticeable differences (JNDs) as a minimum threshold for color discrimination by hummingbirds (Vorobyev et al., 1998). Thus, any contrast higher than 1.00 JND was considered as perceivable by hummingbirds. We used R v. 4.0.2 (R Core Team, 2020) with the additional packages pavo (Maia et al., 2019) and rgl (Adler, 2020) to create and plot the tetrahedron color space model and to calculate the chromatic and achromatic contrasts of the stigma and of the anthers against the corolla, as perceived by hummingbirds.

Floral Scent

We verified if florivory by the most common florivores in nature affects local scent emission in terms of the total amount of floral scent, the relative scent composition, and qualitative scent properties during the flower lifespan. Therefore, we bagged intact pre-anthesis buds to ensure that pollinators or other florivores could not visit them. When the flowers opened, we inserted in one flower per plant a florivore into the bag. We kept the florivores inside the bags, freely feeding on the flowers overnight. In the morning of the next day, we removed them and sampled the VOCs (volatile organic compounds) emitted by the flowers ($n_{damaged}=5$ flowers from 5 different plants). The other flowers ($n_{control}=5$ flowers from 3 different plants), which remained bagged throughout the experiment, served as positive control, and the emitted scents were also sampled in the morning of the second day (**Figure 2**). The florivores used in this experiment were Lycaenidae caterpillars, which were the most common florivores found in this system. We collected caterpillars at third and fourth instars from other individuals of the same population and stored them for a few hours in voile bags before transferring them to the flowers to start the experiment.

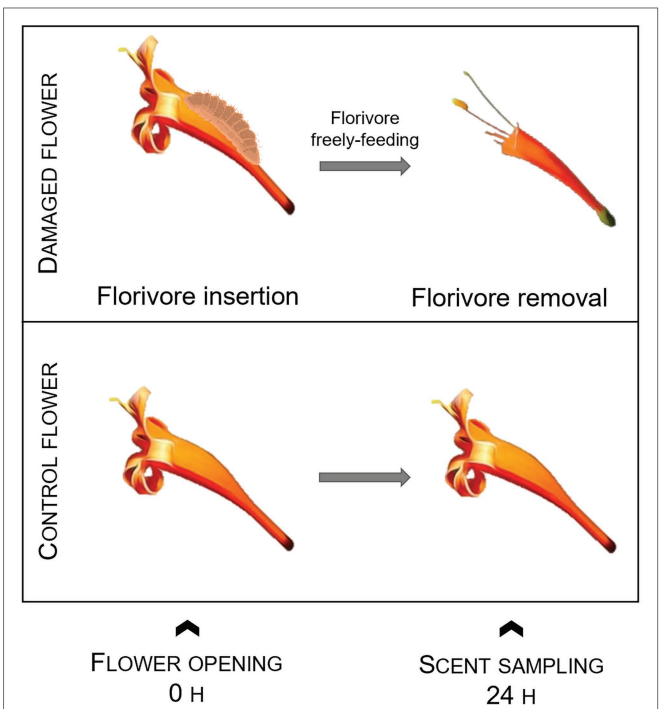

FIGURE 2 | Experimental design used to investigate short-term local changes in floral scent due to florivory by a lycaenid caterpillar. Caterpillar image from Biorender.com.

We collected floral and vegetative (used as negative control) VOC samples following the protocol by Dötterl et al. (2005). We enclosed the flowers or leaves in 12 × 8 cm polyethylene oven bags 10 min before sampling. The VOCs that accumulated inside a bag were collected for 1 h in an adsorbent trap connected to a membrane pump that generated an airflow of 200 ml/min. The adsorbent traps were made from quartz microtubes with approximately 15 mm of length by 2 mm of internal tube diameter. These traps were filled with a mixture of 1.5 mg Tenax-TA (60–80 mesh) and 1.5 mg of Carbotrap B (20–40 mesh, both Supelco). We stored the samples at −20°C until analysis. We analyzed the VOC samples on a TD-20 automated thermo desorption system (Shimadzu) coupled to a QP2010 Ultra EI GC/MS (gas chromatograph coupled to a mass spectrometer, Shimadzu) equipped with a ZB-5 fused silica column (60 m long, 0.25 mm of inner diameter, 0.25 µm of film thickness) and maintained a constant 1.5 ml/min flow of helium as the carrier gas. The injector temperature was 200°C and the samples were injected in split mode 1:3. The oven temperature started at 40°C, then increased by 6°C/min to 250°C, at which it kept constant for 1 min. The MS interface was set at 250°C. Mass spectra were taken at 70 eV (in EI mode), with a scanning range of 30–350 m/z. The data were analyzed using the GCMSolution package, Version 4.41 (Shimadzu). We performed the tentative identification of the volatile compounds present in each sample by comparison of Kovats retention indices (KRI, based on a series of n-alkanes) and mass spectra to data available in the databases ADAMS (Adams, 2007), ESSENTIALOILS-23P, FFNSC 2, Wiley 9 and Nist11. If possible, compound identities were confirmed by authentic reference standards available at the Plant Ecology lab of the Paris Lodron University of Salzburg. Compounds detected in similar amounts in leaf controls and flowers were excluded from the analyses. We only considered compounds that were present in more than twice the amount in flowers than in leaves. For quantitative analysis of VOCs, we injected 100 ng each of *ca.* 150 components, among them monoterpenes, aliphatic, and aromatic compounds, into the GC/MS system. We used the mean of the peak areas (total ion current) of these compounds to estimate the total amount of scent available in the scent samples (Etl et al., 2016).

Floral Resource

To check if florivory affected the amount of nectar, we bagged 80 floral buds 1 day before anthesis from 11 plants ($n = 3$–11 flowers/plant). We assigned each bud to one of two treatments: (i) intact buds, and (ii) buds previously naturally attacked by the main florivore. A single plant contained both treatments. Approximately 12 h after flower opening (*ca.* 18:00 h), we withdrew the accumulated nectar and measured its volume using 50 µl microcapillary tubes (Galetto and Bernardello, 2005).

Effect of the Experimentally Simulated Floral Damages on Hummingbird Pollination

To obtain a large enough sample size to investigate the possible effects of floral damages on hummingbird pollination, we caused the damages manually. Additionally, by causing damages manually we could ensure that all the flowers presented to the hummingbirds showed a standard damage, which would be impossible to guarantee if we used flowers naturally damaged by florivores. However, experimental manipulations do not always incur in the same physiological responses as feeding by natural herbivores (Baldwin, 1990). Therefore, prior to performing this experiment, we controlled for possible effects of experimentally simulated floral damages on floral scent and floral nectar, which are traits that could display a different physiological response to mechanical simulation of damages than that triggered by florivores. For that, we removed the half-upper portion of the corolla tube, replicating the most severe damage caused by the most common natural florivore, a caterpillar (**Figure 1D**). Then, we collected scent samples from manually damaged flowers ($n = 3$ flowers from 3 plants) and compared them to undamaged flowers ($n = 3$ flowers from 3 plants) using the same method described in section 2.3.3. Moreover, we sampled nectar volume of manually damaged flowers ($n = 38$ flowers from 11 plants) and compared them to undamaged flowers ($n = 49$ flowers from 11 plants) using the same method described in section 2.3.4.

We found that experimentally damaged flowers did not differ in their scent from intact flowers [total amount: Pseudo-$F_{(1, 2)} = 1.30$; $p = 0.43$; relative amount: Pseudo-$F_{(1, 2)} = 0.87$; $p = 0.52$; presence/absence: Pseudo-$F_{(1, 2)} = 0.9$; $p = 0.53$], nor in nectar volume ($F = 0.006$; $p = 0.9367$). Therefore, in our study system, manually caused damages did not lead to physiological responses in terms of scent or nectar production.

Additionally, to avoid any possibility of pollination not performed by hummingbirds (Tunes and Guimarães, 2020), we performed the further experiment only with flowers that presented an accentuated approach herkogamy, with the anthers placed below the stigma. We randomly selected 33 plants of the population (3–14 flowers/plant). We submitted the flowers from those plants to one of two treatments: (i) control flowers that were not damaged ($n = 97$ flowers); and (ii) mechanically simulated floral damage, emulating the most serious damage recorded by the most common natural florivore, a caterpillar ($n = 98$ flowers; **Figure 1D**). Each plant contained both treatments in similar amount. We isolated pre-anthesis buds from floral visitors until our manipulations to guarantee that the flowers were all intact at the beginning of the experiment, and randomly assigned the flowers to the two treatments. To experimentally damage the flowers, we used surgical scissors to cut off pieces of the corollas according to the damages naturally caused by Lycaenidae caterpillars (**Figure 1D**). As we were interested in understanding the effects of florivory on plant–pollinator communication and pollination, we focused on corolla damage, because any damage to reproductive structures would definitely affect plant reproductive success. To ensure that control flowers remained intact, we checked these flowers at the beginning and the end of the experiment; flowers that had any damage at the end of the experiment were discarded and replaced in the subsequent days. We performed the experiments during the flowering peak, through 25 consecutive days, when pollinators and flowers were abundant (July–August). To evaluate the likelihood

of a flower receiving hummingbird visits, we used two different and complementary approaches. Initially, we performed focal observations (in person and by video recording) of the pollinator visits ($n = 8$ plants, 30 h, 2–4 h of observation/plant/turn). We registered approximately two visits per plant per hour in the population at the peak of hummingbird visitation, which occurred from dawn until 09:00 h and from 17:00 until dusk. However, this approach did not work well, as visitation to the focal flowers was extremely low. This issue could be due to the fact that hummingbirds actively avoided patches where the observer was located. We needed to position ourselves and the cameras close to the plants (1.5–2 m from them) in order to clearly assess whether hummingbirds visited an intact or damaged flower. Thus, through this method, we could not obtain a robust data set to statistically compare the visits to each treatment.

To overcome this constraint, we additionally evaluated the presence of pollen deposited onto the stigmas of *P. venusta* flowers, which is a reliable proxy of a pollinator visit (Ashman et al., 2020). Therefore, we labeled 195 flowers ($n_{control} = 97$ flowers; $n_{damaged} = 98$ flowers) and exposed them to hummingbird visits during the 2 days of anthesis. Following corolla abscission, we collected the stigmas, fixed them in FPA solution (formalin 40%, concentrated propionic acid, ethanol 50%, in volumes of 5:5:90), and stained them with aniline blue and potassium acetate, following the protocol proposed by Dafni et al. (2005). Then, we evaluated the presence/absence of pollen grains adhered to the stigmas' surfaces under a fluorescence microscope (Zeiss Axioskop 40 Microscope, with AxioVision 4.7.2) equipped with a filter set (of maximum transmission at 365 nm).

Statistical Analysis

We used GLMM with gamma error distribution to evaluate if the removal of a portion of the corolla, due to florivory, lead to a change in the chromatic and achromatic contrasts of the anthers and the stigma against the remaining portion of the corolla, considering the interaction between type of reproductive structure (anthers or stigma) and treatment (before or after damage). Plant and flower were used as random variables. We performed a PERMANOVA (9,999 permutations) to evaluate if the total and relative amounts of floral scent as well as qualitative scent properties differed among treatments (control and damaged flowers), considering plant individual as a random factor, based on Euclidean distances for the total amount of scent, on Bray–Curtis dissimilarities for the relative amount of scent, and on Sørensen dissimilarities for qualitative scent properties (presence and absence of compounds). These three different scent characteristics were analyzed as they all might influence floral visitor choices. We performed all PERMANOVA analyses using Primer 6 v. 6.1.15 with PERMANOVA+ v. 1.0.5. We verified if florivory affected nectar volume using GLMM with gamma error distribution, considering treatment as a fixed variable and individual plant as a random variable. We carried out both GLMMs with gamma error distribution in R v. 4.0.2 (R Core Team, 2020) with additional packages: car (Fox and Weisberg, 2019), emmeans (Lenth, 2020), fitdistrplus (Delignette-Muller and Dutang, 2015), ggplot2 (Wickham, 2016), glmmADMB (Fournier et al., 2012; Skaug et al., 2016), lattice (Sarkar, 2008), lme4 (Bates et al., 2015), MASS (Venables and Ripley, 2002), R2admb (Bolker et al., 2020), and viridis (Garnier, 2018). The probability of pollen grains (binary variable) being deposited onto the stigmas was modelled, using GLMM with binomial error distribution, again considering treatment as a fixed variable and individual plant as a random variable. This GLMM was carried out in R v. 4.0.2 (R Core Team, 2020) with standard and additional packages: lme4 (Bates et al., 2015), and nlme (Pinheiro et al., 2018).

RESULTS

Natural Incidence of Florivory and the Florivores Associated to It

We sampled 935 flowers of *Pyrostegia venusta* (Bignoniaceae), from which 77.4% did not present any damage, while 22.6% showed variable degrees of corolla tissue removal. The most common damage corresponded to the category of 1–3% of corolla removal (registered in 14% of the flowers), followed by the 4–5% of corolla removal (registered in 3.7% of the flowers), then by the 6–10% of corolla removal (1.9% of the flowers) and by the other five categories, corresponding to larger damages, which comprised the remaining 3% of the flowers (less than 1% of the flowers per category).

The grasshopper *Schistocerca flavofasciata* (yellow-lined grasshopper, Acrididae) consumed the medium dorsal portion of the corolla tube (**Figures 3A,B**) and small *Trigona* bees made holes in the corolla tube of floral buds, near the portion where the anthers were placed. Both florivores removed 1–3% of corolla tissues. Caterpillars of *Parrhasius polibetes* (black-spot hairstreak, Lycaenidae; **Figure 3C**) were the most common florivore, corresponding to *ca.* 90% of all the observed florivores in the field. They feed on the corolla and on floral reproductive structures. We observed in the field these caterpillars causing floral damages that belonged to every category, from the category of 1–3% until the category of 51–60% of corolla removal.

Effect of Florivory on Floral Traits

Floral Outline

The only florivore that affected floral outline was the lycaenid caterpillar. Larvae of these butterflies consumed from small to large amounts of the corolla, including the lobes and upper portion of the corolla tube. Therefore, they changed the corolla outline, especially, when we consider the most pronounced levels of florivory, which led to the complete removal of the petal lobes in approximately 0.5% of the flowers (**Figures 1D**, **4**).

Intra-floral Color Pattern

The flowers presented no UV reflection/absorption pattern and were UV-absorbing (**Supplementary Figure 2**). Therefore, the only intra-floral color pattern present in these flowers was the human-visible pattern between the reproductive structures and the corolla. The chromatic and achromatic contrasts between the anthers/stigma and the corolla lobes in intact flowers are similar to the chromatic and achromatic contrasts between the anthers/stigma and the remaining lower half of the corolla in

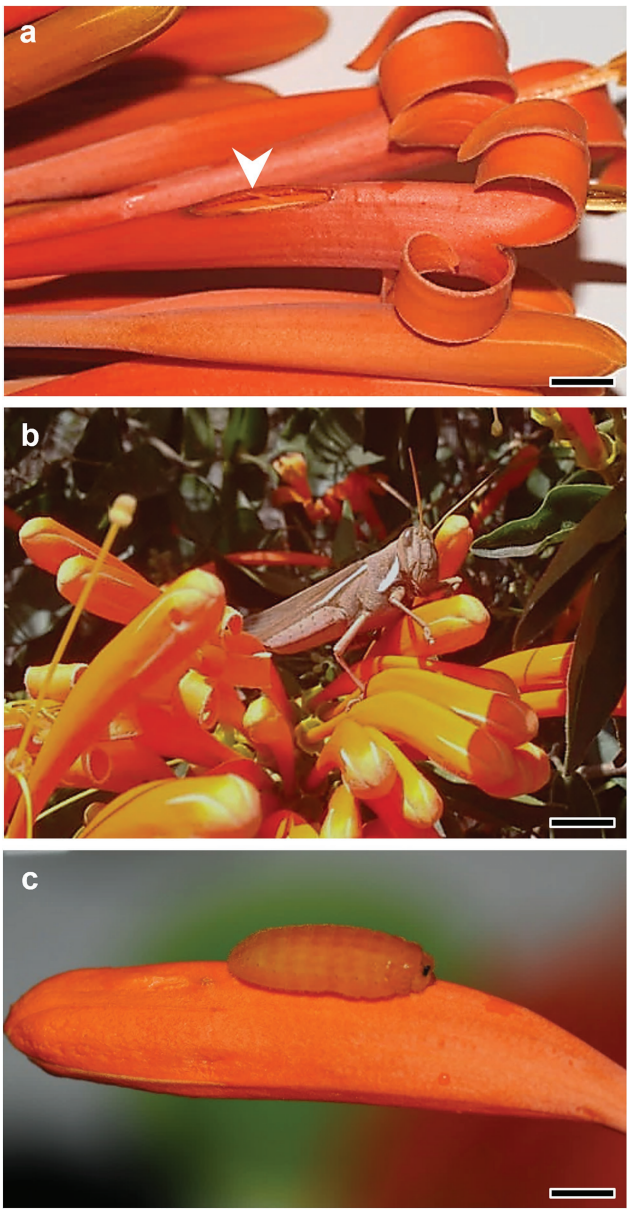

FIGURE 3 | *Pyrostegia venusta* and its florivores. **(A)** Natural floral damage (arrowhead) with 1–3% of corolla damage. Scale bar: 0.7 cm. **(B)** *Schistocerca flavofasciata* (yellow-lined grasshopper, Acrididae) which consumed the flowers leaving bite marks as shown in **Figure 1E**. Scale bar: 1.3 cm. Photograph by N. M. Gildo. **(C)** *Parrhasius polibetes* (black-spot hairstreak, Lycaenidae) caterpillar, which was the most common florivore in *P. venusta*. Scale bar: 0.4 cm.

damaged flowers (**Figure 5**; $p > 0.05$; see **Supplementary Table 1** for mean values and **Supplementary Table 2** for statistical details).

Floral Scent

Intact flowers and flowers that were damaged by florivores (category of 51–60% of corolla removal) emitted a similar amount of scent [Pseudo-$F_{(1,\ 2.12)} = 2.26$; $p = 0.30$; **Table 1**] and had a similar qualitative scent pattern [Pseudo-$F_{(1,\ 2.06)} = 3.84$; $p = 0.11$]. Both treatments emitted mainly aromatic compounds

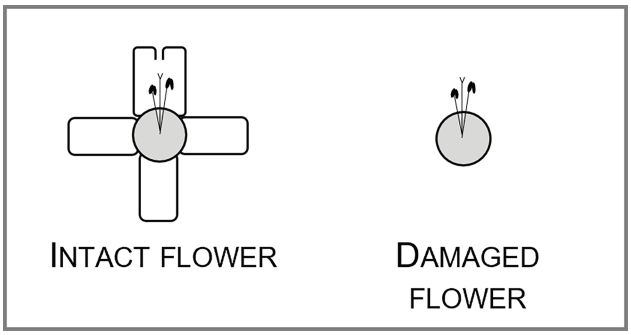

FIGURE 4 | Schematic representation of the most pronounced damage caused by a lycaenid caterpillar florivores. The floral tube, the five corolla lobes, stamens and style are represented.

and terpenoids. These compounds were emitted in similar relative amounts by flowers of the two treatments [PERMANOVA "Treatment"; Pseudo-$F_{(1,\ 2.06)} = 2.06$; $p = 0.20$; **Figure 6**]. On average, the most abundant compounds were β-bourbonene as well as β-caryophyllene in control flowers, benzaldehyde in damaged flowers, and 4-methylanisole in both treatments. It is noteworthy that relative scent properties were, independent of the treatment, highly variable among plants (**Table 1**; **Figure 6**).

Floral Resource

We found no difference when comparing control (39.2 ± 21.6 µl, mean ± sd) and naturally damaged (37.3 ± 21.8 µl) flowers regarding nectar volume (**Figure 7**; $F = 0.916$; $p = 0.3384$).

The Effect of Experimentally Simulated Floral Damages on Hummingbird Pollination

Our beforementioned results evinced that natural florivory did not affect intra-floral color pattern, floral scent, or nectar production. These results validate the use of mechanically damaged flowers (which did not alter those floral traits either) to evaluate the outcomes of florivory on hummingbird pollination.

We recorded the hummingbirds *Phaethornis pretrei* (Phaethornithinae), *Chlorostilbon lucidus* (Trochilinae), and *Polytmus guainumbi* (Trochilinae) as pollinators of *P. venusta*. All of them always contacted both male and female reproductive structures while visiting control and damaged flowers. We did not observe a difference when comparing the likelihood of pollen grains being deposited onto the stigmas of intact and damaged flowers ($Z = 0.279$, $p > 0.1$, $n_{flowers} = 195$, $n_{plants} = 33$), with 47.07 ± 74.37 (mean ± sd) pollen grains found in the stigmas of control flowers and 44.56 ± 75.22 in damaged flowers.

DISCUSSION

In natural populations, the majority of *P. venusta* flowers (77.4%) was intact and the remaining 22.6% of the flowers showed variable natural levels of floral damage, being a Lycaenidae caterpillar the most frequent florivore observed in the system. This florivore

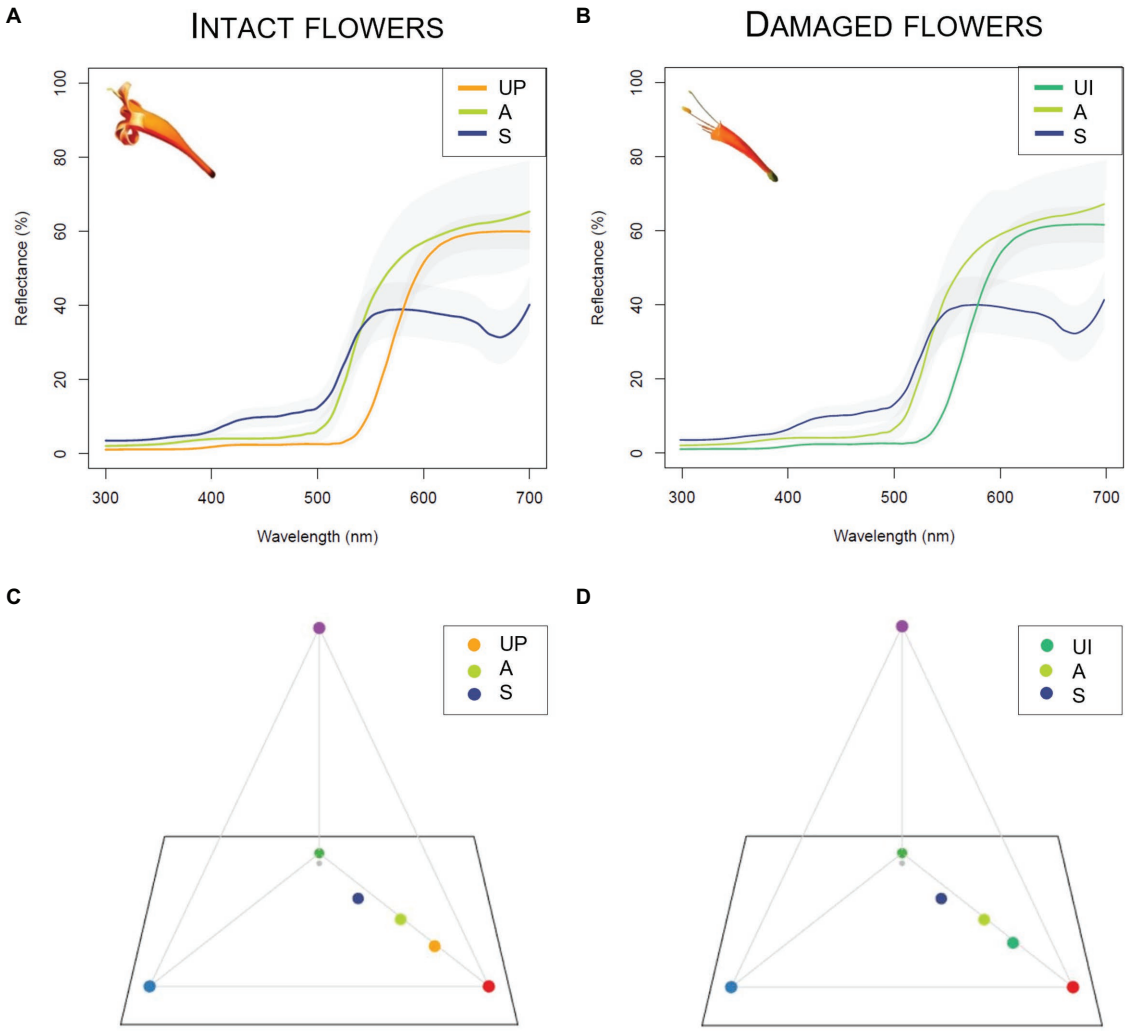

FIGURE 5 | Spectral reflectance of intact and damaged *Pyrostegia venusta* (Bignoniaceae) flowers ($n = 20$ flowers from 8 plants) and colors modelled using the tetrahedron vision model for birds. **(A,B)** Spectral reflectance curves. The lines represent the mean reflectance of the upper petal lobe (UP), the upper internal portion of the corolla tube (UI), the anthers (A) and the stigma (S). The shaded areas represent the standard variation of reflectance measures. **(C,D)** Tetrahedron vision model for birds. The central grey dots represent the achromatic center; the colored dots represent the mean loci for the aforementioned portions of the flowers. The maximum excitation of the ultraviolet, blue, green, and red photoreceptors is represented by the respective tetrahedron vertices (violet, blue, green, and red).

caused damages, which were variable in size and shape, fitting the categories from 1–3% to 51–60% of corolla removal. Despite the accentuated loss of corolla integrity caused by this florivore, floral color patterns, floral scent locally emitted by damaged flowers and floral nectar volume were not affected. Mechanical simulation of the change in corolla outline caused by caterpillars did not affect pollination by hummingbirds (see a graphical summary of the main findings of this study in **Figure 8**).

Pyrostegia venusta presents a massive visual floral display, with synchronous production of numerous orange short-lived flowers (Gobatto-Rodrigues and Stort, 1992). This massive visual advertisement will not only be perceived by pollinators, but also by florivores (Anderson, 1996; Harder and Johnson, 2005). Indeed, not only hummingbirds, but also grasshoppers and butterflies (which lay eggs inside the flowers that develop into caterpillar florivores), are capable of perceiving orange flowers (Vishnevskaia and Mazokhin-Porshniakov, 1969; Chittka and Briscoe, 2001 and references therein; Vanhoutte, 2003; Briscoe, 2008). Thus, one can expect high levels of florivory due to the local abundance of resources. Similarly, to our study system, Kessler et al. (2013) also found a prevalence of natural low levels of floral tissue removal performed by orthopterans. High levels of florivory, comparable in size to the largest damages observed here, also performed by lepidopteran caterpillars, were described for a bee-pollinated system (McCall, 2008).

Regarding floral visual advertisements, we could expect at least two types of effect of florivory. The first and more obvious

TABLE 1 | Total absolute (mean ± sd; minimum–maximum; ng. flower-1. hour-1) and relative amount of each compound (mean ± sd; minimum–maximum; %) of scent in intact and naturally damaged flowers of *Pyrostegia venusta*.

		Intact flowers	Naturally damaged flowers
$n_{flowers}$ (n_{plants})		5 (3)	5 (5)
Total number of compounds		23	18
Mean total amount of scent		43.1 ± 39.80 (12.6–112.7)	30.1 ± 36.93 (2.7–94.4)
Compound class	**RI**		
Aliphatic compounds			
Hexanal*	798	2.37 ± 3.81 (0–8.76)	8.21 ± 12.58 (0–28.51)
(*E*)-2-Hexenal*	852	0.46 ± 0.65 (0–1.39)	-
Aromatic compounds			
Benzaldehyde*	963	8.46 ± 18.91 (0–42.3)	33.96 ± 31.85 (0–76.59)
4-Methylanisole*	1,025	12.03 ± 15.06 (0.82–37.6)	18.81 ± 29.04 (0.23–70.21)
Benzyl alcohol*	1,036	3.59 ± 8.04 (0–17.98)	2.79 ± 4.4 (0–10.31)
2-Phenylethanol*	1,118	1.13 ± 1.8 (0–4.14)	–
Methyl salicylate*	1,204	–	3.61 ± 7.25 (0–16.54)
Terpenoids			
6-Methyl-5-hepten-2-one*	987	4.25 ± 5.92 (0–12.15)	5.27 ± 5.52 (0–12.86)
Limonene*	1,036	2.26 ± 4.24 (0–9.76)	0.19 ± 0.42 (0–0.95)
α-Copaene*	1,395	3.31 ± 3.32 (0–7.53)	1.39 ± 3.12 (0–6.99)
β-Bourbonene*	1,407	16.35 ± 10.1 (0–26.75)	5.14 ± 11.51 (0–25.74)
β-Caryophyllene*	1,445	14.67 ± 30.22 (0–68.62)	6.41 ± 14.34 (0–32.07)
α-Caryophyllene*	1,481	5.03 ± 11.25 (0–25.16)	1.98 ± 4.42 (0–9.9)
Valencene*	1,515	4.12 ± 5.65 (0–10.62)	0.55 ± 1.23 (0–2.75)
(*E*)-Nerolidol*	1,571	0.52 ± 0.54 (0–1.27)	0.16 ± 0.35 (0–0.8)
1-*nor*-Bourbonanone	1,588	5.61 ± 5.5 (0–11.2)	4.72 ± 6.49 (0–12.58)
Unknown compounds			
m/z: 43.82.67.55.41.83	1,292	0.4 ± 0.55 (0–1.08)	–
m/z: 57.161.105.43.119.83	1,389	0.7 ± 0.97 (0–1.97)	–
m/z: 161.120.105.91.43.55	1,441	2.58 ± 1.54 (0–3.95)	0.99 ± 1.7 (0–3.93)
m/z: 161.105.91.119.79.133	1,452	2.35 ± 3.41 (0–7.47)	1.17 ± 2.62 (0–5.86)
m/z: 105.93.79.121.161.204	1,513	2.43 ± 5.44 (0–12.17)	3.44 ± 7.69 (0–17.2)
m/z: 41.121.55.206.163.93	1,638	1.96 ± 2.68 (0–5.11)	–
m/z: 43.108.93.126.41.71	1706	4.35 ± 6.77 (0–15.44)	1.13 ± 1.83 (0–4.21)
m/z: 43.91.121.79.107.135	1741	0.98 ± 1.57 (0–3.61)	–

Scent compounds are listed according to their chemical class and Kovats' Retention Index (RI) based on a series of n-alkanes (C6–C20). Compounds marked by an asterisk were identified based on their mass spectra and the RI of synthetic standards.

one is the loss of the physical integrity of the corolla. Although after florivory the remaining portion of the corolla is still tubular, the general outline of the flower was modified. Hummingbirds perceive floral shapes and those with short straight bill prefer shorter and straighter floral tubes, whereas hummingbirds with long curved bills prefer longer and more curved flowers (Maglianesi et al., 2015). Therefore, as hummingbirds present this capability of distinguishing floral shapes, especially regarding floral tube length, the damages caused by florivores could lead hummingbirds not to recognize the "new" floral outline as that originally associated with the trophic resource (intact flowers). Nevertheless, contrary to this expectation, our results show that hummingbirds did not reject damaged flowers. A second expected effect of florivory on floral visual advertisements is changes in intra-floral color pattern. Florivory did not affect intra-floral color patterns, as the chromatic and achromatic contrasts between the yellowish reproductive structures and the orange corolla did not differ between intact and damaged flowers. Overall, florivory in *P. venusta* changed floral outline but preserved intra-floral color patterns, with no effects on pollination success. This contrasts with the findings for *Mimulus luteus*, another hummingbird-pollinated species, in which florivory changed color patterns, with negative effects on pollination (Pohl et al., 2006).

Regarding floral chemical advertisements, hummingbird-pollinated flowers are commonly regarded as being scentless or present few and widespread VOCs (Knudsen et al., 2004). However, we found 24 scent compounds in *P. venusta*, which is up to 8.3 times greater than what had been previously described for other hummingbird-pollinated species (Knudsen et al., 2004). Among these compounds are several that are also known from other hummingbird-pollinated plants (Knudsen et al., 2004; Dellinger et al., 2019). Some are even behaviorally active in hummingbird species [attractants: benzaldehyde, (E)-β-caryophyllene; repellents: limonene, methyl salicylate; see Kessler and Baldwin (2007)] and might also be involved in the communication between *P. venusta* and their hummingbird pollinators. Similarly to the number of compounds, also the total amount of floral scent is highly variable among hummingbird-pollinated species, also showing high levels of intra-specific variation (Dellinger et al., 2019), as we recorded in *P. venusta*. The ecological consequences of intra-specific scent variation in hummingbird-pollinated

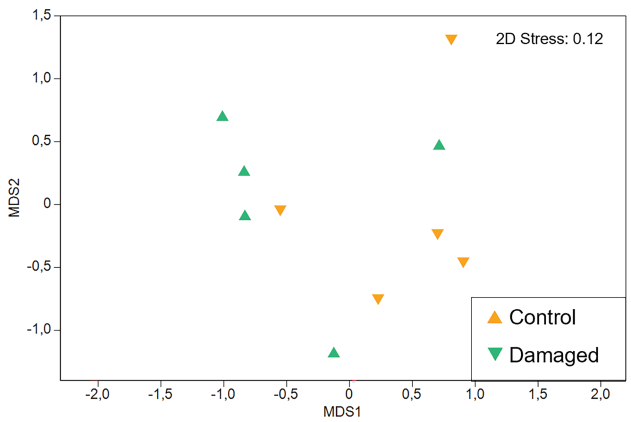

FIGURE 6 | Non-metric multidimensional scaling (NMDS) of *Pyrostegia venusta* floral scent from control and naturally damaged flowers. Comparison between the relative scent composition emitted by flowers that were intact (control) or naturally damaged by the florivore caterpillar, *Parrhasius polibetes* (black-spot hairstreak, Lycaenidae). There was no significant difference in relative scent composition among flowers submitted to both treatments (PERMANOVA with 9,999 permutations, Pseudo-$F_{[1, 2.06]} = 2.06$; $p = 0.20$). Each dot represents a sample.

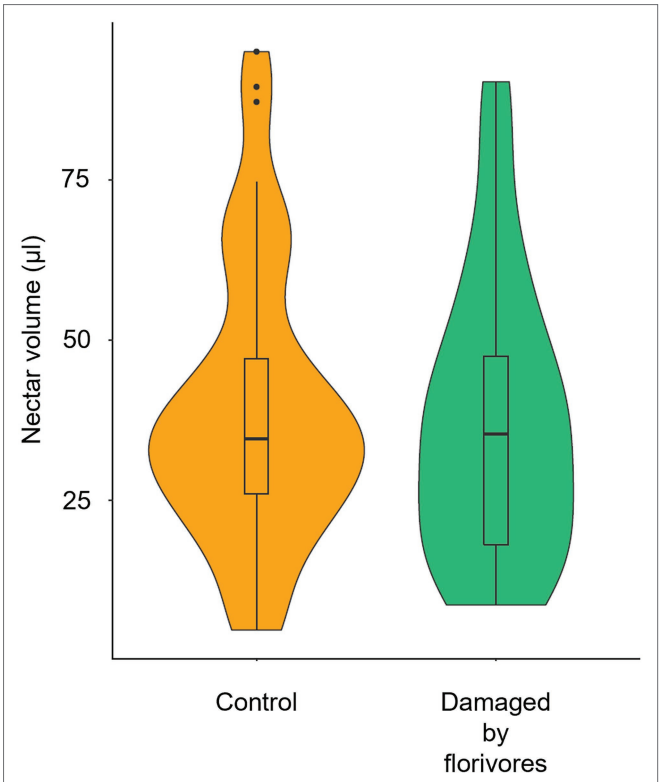

FIGURE 7 | Violin plots of nectar volume in *Pyrostegia venusta* flowers that were intact (control) and damaged by florivores. The median, the 25th and 75th percentiles, the non-outlier range (within 1.5 times the interquartile range), and the outliers are shown. There was no significant difference among the nectar volume in flowers submitted to the different treatments (GLMM with gamma error distribution, $p = 0.3384$).

species are unknown so far, but are currently under investigation (Guimarães et al., unpublished data). Regardless, as the amount of scent emitted by *P. venusta* flowers and its relative chemical composition were similar in intact and damaged flowers, one can infer that both types of flowers, based on the olfactory advertisement, are indistinguishable to hummingbirds.

Although visual and chemical advertisements are determinant for flower location by hummingbirds, a reliable nectar supply will be essential for maintaining hummingbird visits to the flowers (George, 1980; Irwin, 2000; Altshuler and Nunn, 2001; Hurly and Healy, 2002; Chautá et al., 2017). Damages inflicted by florivores to *P. venusta* flowers did not affect the amount of nectar available per flower, differently from shown by Radhika et al. (2010) for *Brassica napus*. Therefore, by operant conditioning, with nectar acting as positive reinforcement, hummingbirds can easily learn to keep visiting *P. venusta* flowers, even in the presence of severe damages.

In general, most studies that evaluated the effect of florivory on plant fitness have found negative effects; however, this result is vastly variable among plant species (see Haas and Lortie, 2020 for references). These negative effects are associated with pollen limitation (Leavitt and Robertson, 2006; McCall, 2010), or a specific decrease in plant attraction to bees (Karban and Strauss, 1993; Krupnick et al., 1999; McCall, 2008), bats (Von Helversen and Von Helversen, 1999) and hawkmoths (Mothershead and Marquis, 2000). Regarding hummingbird-pollinated systems, the studies have shown contrasting results. Our experimental approach revealed that florivory did not affect *P. venusta* pollination, as found by (Tsuji et al., 2016). However, in other systems, it negatively altered plant fitness, especially, when florivory affected floral guides (Pohl et al., 2006). It is possible that the mechanisms underlying these variable outcomes, which are poorly known, may be species-specific from plant, pollinator, and florivore perspectives (Rusman et al., 2019b).

Moreover, the ecological consequences of florivore-induced local short-term changes on plant–pollinator interactions may be substantially distinct from those of systemic responses. Some of the effects of florivory are only expressed locally, such as changes in flower shape, outline and size, or changes in the intra-floral color patterns, as direct "byproducts of florivore action." However, other effects, such as volatile emission, pigment allocation, and nectar features, can change due to a local or a systemic response, as a "plant reaction to florivore action." Local effects of florivory should be fast enough to be expressed during the flower lifetime. On the other hand, systemic effects may be fast or not and will comprise the whole plant, including damaged and undamaged flowers (Rusman et al., 2019a). In our study, we found no differences in most of the traits evaluated, except for the inevitable change in the flower outline due to the removal of corolla portions by the florivores, which is a local direct effect of florivory. From the pollinator perspective, the flower is the unit that must be recognized when searching for resources. Thus, the short-term local effects of florivory have

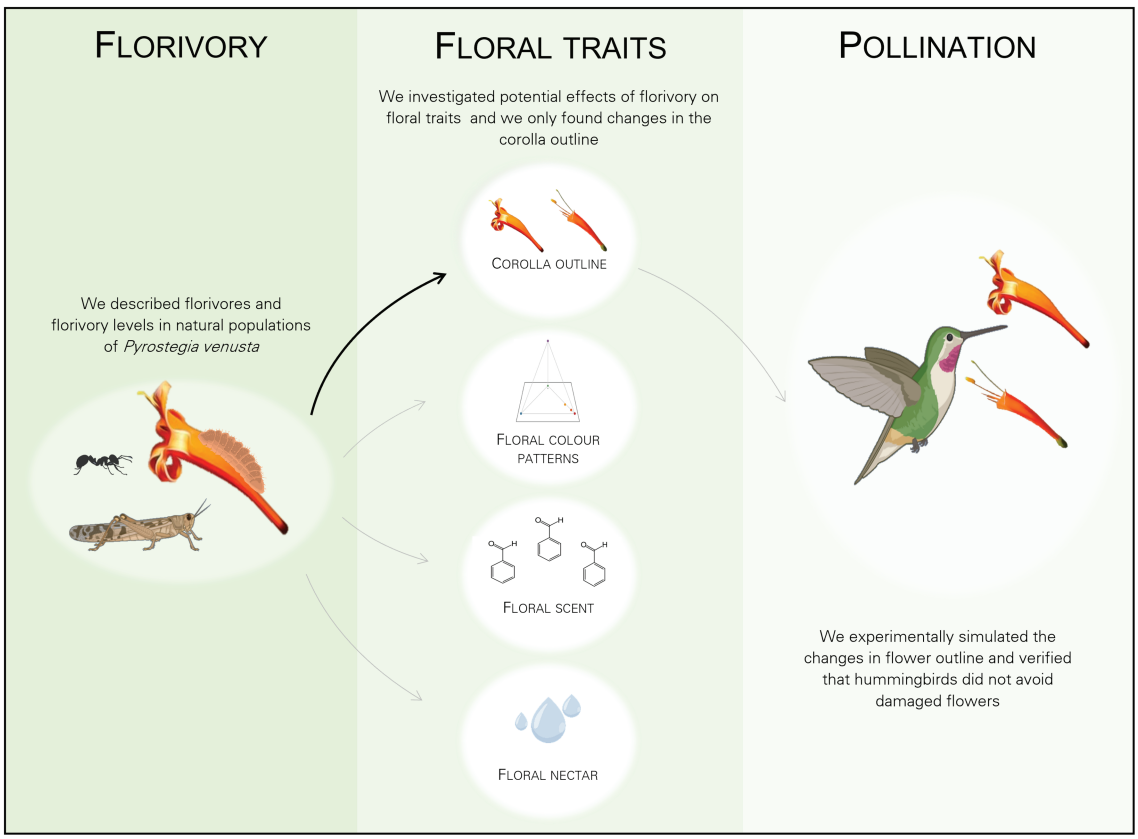

FIGURE 8 | Graphical summary of the experimental design of the study showing the general design and main results. Florivory, by the most common florivore, a Lycaenidae caterpillar, did not affect floral color patterns, nor floral scent, nor floral nectar volume (grey arrows). Florivory only affected flower integrity, by changing the corolla outline due to the presence of damages *per se* (black arrow). Finally, we show that there was no effect of the mechanical simulation of floral damages on pollination (grey arrow). Created with BioRender.com.

a large potential to immediately interfere with flower–pollinator communication pathways.

There is a growing body of literature regarding the effects of florivory on pollination (Soper Gorden and Adler, 2016, see Haas and Lortie, 2020 for references) and the present study simultaneously investigated the local effects of florivory on visual and olfactory advertisements, as well as on floral sugar resource. Considering all our data, we have shown that florivory only led to the loss of corolla integrity due to the presence of damages *per se*, which changed corolla outline. However, during the flower lifespan, florivory did not have effects on intra-floral color patterns, floral scent, or floral nectar. Thus, this study isolates a single effect of florivory—floral outline modification—and demonstrates that this isolated change in flower appearance does not discourage hummingbird visitation, even though hummingbirds strongly rely on vision for food location (Hickman and Robert, 1993; Pritchard et al., 2017; Tyrrell et al., 2018 and references therein). Thus, this study highlights that the florivore–plant–pollinator intersection may work as a complex and stable trophic system, in which local changes in floral traits promoted by florivory are not enough to disrupt flower–hummingbird communication and pollen transfer itself.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

PT and EG conceived the study, collected and analyzed the data, and wrote the main manuscript text. SD contributed to the scent analyses and commented on earlier versions of the manuscript. All authors contributed critically to the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.813418/full#supplementary-material

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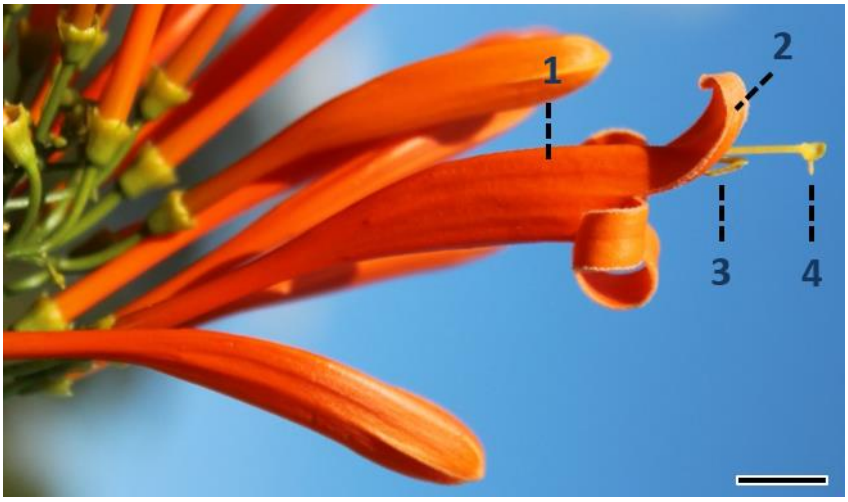
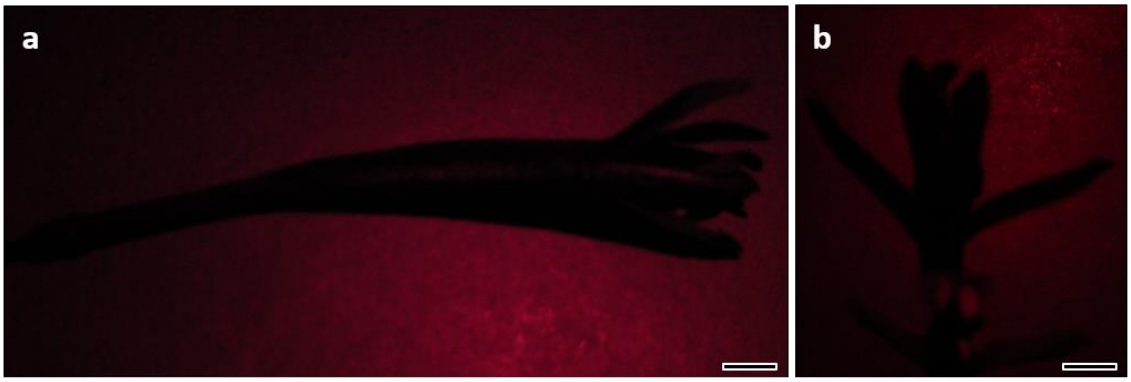
Supplementary Material



Supplementary Figure 1. Floral portions of *Pyrostegia venusta* in which we measured the spectral reflectance. 1: upper internal portion of the corolla tube (UI); 2: upper petal lobe (UP); 3: anthers (A); 4: stigma (S). Scale bar: 0.9 cm.



Supplementary Figure 2. *Pyrostegia venusta* (Bignoniaceae) flower photographed using a camera with a modified sensor that only captures UV light and illuminated by an UV light source. Surfaces that reflect UV light become bright red in the image. (A) Side view; **(B)** Frontal view. Scale bar: 0.5 cm. Note that, in both views of the flower, we cannot observe any portion of the flower in red, which indicates that the whole flower absorbs UV-light.

Supplementary Table 1. Mean and standard deviation of the chromatic and achromatic contrasts between floral parts in Just Noticeable Differences (JNDs) of *Pyrostegia venusta* flowers (n = 20 flowers from eight plants). The contrasts of both reproductive structures against the upper petals correspond to the contrasts observed in intact flowers, and the contrasts against the upper internal portion of the corolla tube correspond to the contrasts observed in damaged flowers.

Treatment	Contrasting floral parts	Chromatic contrast	Achromatic contrast
Control	Anthers - Upper petal lobes	6,32 ± 2,06	3,84 ± 2,22
	Stigma - Upper petal lobes	12,07 ± 2,13	2,6 ± 1,74
Damaged	Anthers - Upper internal portion of the corolla tube	5,68 ± 1,72	3,7 ± 2,02
	Stigma - Upper internal portion of the corolla tube	11,23 ± 2,55	2,42 ± 1,8

Supplementary Table 2. GLMM results, based on the models "chromatic contrast ~ treatment + contrasting parts + (1|plant) + (1|flower)" and "achromatic contrast ~ treatment + contrasting parts + (1|plant) + (1|flower)". A = anthers, S = stigma, SE = standard error.

Type of Contrast	'Treatment' effect					'Contrasting parts' effect				
	Comparison	Coefficient	SE	Z value	p-value	Comparison	Coefficient	SE	Z value	p-value
Chromatic	A Control-Damaged	-0.0085	0.0053	-1.595	0.1107	Control A-S	0.0797	0.0066	12.166	**<.0001**
	S Control-Damaged	-0.0085	0.0053	-1.595	0.1107	Damaged A-S	0.0797	0.0066	12.166	**<.0001**
Achromatic	A Control-Damaged	-0.0147	0.0497	-0.296	0. 7670	Control A-S	-0.1283	0.0543	-2.364	0. 0181
	S Control-Damaged	-0.0147	0.0497	-0.296	0.7670	Damaged A-S	-0.1283	0.0543	-2.364	0.0181

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CONSIDERAÇÕES FINAIS

Através deste estudo, verificamos que, de modo geral, a florivoria resultou em poucos e pequenos danos em espécies vegetais de cerrado. Sendo que a florivoria sofrida por uma espécie vegetal não é modulada por sua distribuição espacial, nem pela distribuição espacial de suas unidades de atração, nem pelo tamanho de suas unidades de atração, nem com base em suas relações filogenéticas, mas está associada ao tipo de unidade de atração apresentado pelas espécies vegetais. Os fatores que podem estar contribuindo para os baixos níveis de florivoria observados podem estar associados a distintos mecanismos de escape aos florívoros, à especificidade de sinalização das flores aos polinizadores, ou ainda à presença de compostos defensivos nas flores. Adicionalmente, diversos desses fatores podem atuar concomitantemente, moldando o cenário natural descrito neste estudo.

Além de buscarmos identificar os fatores moduladores da florivoria em escala de comunidade, buscamos compreender os efeitos da florivoria sobre potenciais atrativos florais que atuam nas vias de comunicação flor-polinizador, integrando aspectos visuais e químicos das relações florívoro-planta-polinizador. Esses aspectos são essenciais para a compreensão da dinâmica dessas interações, bem como para a determinação das consequências da ocorrência de uma interação no sucesso da outra. Neste sentido, o presente estudo representa mais um passo em direção ao entendimento de potenciais influências da florivoria sobre os caracteres florais utilizados pelos polinizadores para localizar e visitar as flores.

Nossos dados apontam para a existência de efeitos espécie-específicos da florivoria sobre a quantidade total de voláteis florais emitidos após os danos. Entretanto, na maioria dos casos, a florivoria não afetou o odor floral, indicando que a comunicação química entre planta e polinizador pode ser substancialmente estável. Adicionalmente, constatamos que a simetria floral, a área da superfície floral e a integridade de sua forma não são essenciais para a manutenção da interação planta-polinizador. Assim, concluímos que, de modo geral, a florivoria apresenta efeitos muito mais moderados e sutis sobre os caracteres florais do que os reportados na literatura sobre o efeito da herbivoria foliar. Adicionalmente, por a florivoria ser uma interação bem estabelecida desde o início do Período Cretáceo, precedendo até mesmo a interação de polinização, é possível que o sistema florívoro-flor-polinizador apresente uma grande estabilidade, sendo menos

responsivo do que se supõe, permitindo assim a manutenção desses sistemas complexos com pouca ou nenhuma consequência negativa para a reprodução das espécies vegetais.