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NAYARA SILVA LINS DE ALBUQUERQUE

SINAIS VISUAIS E OLFATIVOS NA ATRAÇÃO DOS POLINIZADORES DE FLORES DE ÓLEO

RECIFE

2021

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Tese apresentada ao Programa de Pós-graduação em Biologia Vegetal da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de doutor em Biologia Vegetal. Área de concentração: Ecologia e Conservação.

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Em memória da minha avó, Maria Alves, com quem aprendi a admirar a beleza das flores.

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RESUMO

Os sinais visuais e olfativos florais atuam na atração dos polinizadores. A presença de determinadas características pode definir qual espécie de polinizador vai ser atraída pela flor. Esses sinais são percebidos de maneira diferente pelos diferentes polinizadores, pois cada grupo de animais possuem sistemas sensoriais diferentes. Essa tese foi realizada usando como modelo um conjunto de espécies de plantas que produzem óleo como recurso floral, sistema que é bastante especializado. Os objetivos foram caracterizar os sinais visuais e olfativos de flores neotropicais de espécies de flores de óleo e investigar o padrão de cor de espécies que mudam a coloração das suas flores. Para isso, foram realizadas coletas de dados em espécies de diferentes domínios fitogeográficos: Caatinga, Mata Atlântica e Campo Rupestre. Com o auxílio de um espectrofotômetro, foram mensurados os espectros de cor de flores de 11 espécies de flores de óleo (Malpighiaceae, Plantaginaceae, Krameriaceae e Iridaceae). Para caracterizar o odor floral foram coletadas amostras de extratos florais (flores mergulhadas em hexano) de 14 espécies (Malpighiaceae, Plantaginaceae e Krameriaceae) e analisadas a partir da técnica de Cromatografia gasosa acoplada a espectrometria de massas (GCMS). *Banisteriopisis muricata* e *Byrsonima gardneriana* (Malpighiaceae) foram usadas como modelos para investigar a mudança de coloração de flores ao longo da antese. As espécies neotropicais estudadas mostram uma grande diversidade de padrões de cores e odores florais, sendo a composição dos odores florais espécie específica e com pouca/nenhuma relação ao gênero ou família da espécie de flor de óleo analisada. Essa diversidade de padrões visuais e olfativos pode refletir na diferença de composição das espécies visitantes de abelhas coletoras de óleo, como é observado na natureza. As mudanças de cor das flores durante a antese são evidentes no hexágono de visão das abelhas. Portanto, essas mudanças de cores parecem estar relacionadas a sinalização do recurso floral. Os objetivos do segundo artigo foram comparar os sinais visuais e olfativos de dois morfos de *Byrsonima sericea* (com e sem glândulas de óleo) e comparar a composição dos visitantes florais e o sucesso reprodutivo desses diferentes morfos. Os mesmos equipamentos utilizados no primeiro artigo para análises de cor e odor foram usados no segundo. Coletamos dados de cor de diferentes partes florais dos dois morfos. Também foram coletadas amostras de extrato e de *headspace* do odor floral dos diferentes morfos, que foram analisadas por GCMS. O padrão de cor e o perfil de voláteis florais são semelhantes nos dois morfos. No entanto, existem compostos mais pesados, apenas identificados nos extratos de odores florais das flores com glândulas. Esses compostos parecem ser oriundos do óleo e, devido à sua baixa volatilidade, devem ser percebidos pelas abelhas apenas a curta distância. As observações de campo indicam que não há diferença na composição dos visitantes florais e que o sucesso reprodutivo dos dois morfos são iguais. Portanto, os sinais visuais e olfativos à longa distância parecem enganar os polinizadores e garantir o sucesso reprodutivo também do morfo sem glândulas de óleo.

Palavras–chave: Cor floral; odor floral; flores de óleo; abelhas coletoras de óleo; engodo.

ABSTRACT

Floral visual and olfactory signals act in attracting pollinators. The presence of certain traits can define which species will be attracted to the flower. These signals are perceived differently by different pollinators, because each group of animals has different sensory systems. Our study was done using as a model a set of plant species that produce oil as a floral resource, a system that is quite specialized, with the bees of the tribes (Centridini, Tapinotaspidini, and Tetrapediini) exclusively as pollinators. The aims of the first article were to characterize the visual and olfactory signals of neotropical flowers of oil-flower species and to investigate the color pattern of species that change the coloration of their flowers. For this purpose, data collections were made in species from different regions: Caatinga, Atlantic Forest and Campo Rupestre. To characterize the visual signals, the color spectra of flowers of 11 species (Malpighiaceae, Plantaginaceae, Krameriaceae and Iridaceae) were measured with a spectrophotometer. To know the floral scent profile, samples of floral extracts from 14 species (Malpighiaceae, Plantaginaceae and Krameriaceae) were collected and analyzed with GCMS (Gas chromatography coupled to mass spectrometer). *Banisteriopisis muricata* and *Byrsonima gardneriana* (Malpighiaceae) were used as models to investigate the change of flower coloration throughout anthesis. The Neotropical species studied show a great diversity of color and floral scent patterns, the composition of floral scents is species specific and with little/no relation to the genus or family of the oil flower species analyzed. This diversity of visual and olfactory patterns may reflect in the difference in composition of the visiting species of oil collecting bees. The color changes of the flowers are evident in the hexagon of the bees' vision; these color changes seem to be related to flower resource signaling. The objectives of the second article were to compare the visual and olfactory signals of two morphs of *Byrsonima sericea* (with glandular flowers and one with engladular flowers) and to compare the composition of floral visitors and the reproductive success of these different morphs. The same equipment used in the first article to analyses the color and scent the flowers was used in the second. We collected color data from different floral parts of the two morphs, with and without oil glands. Extract and headspace samples of floral odor from the different morphs were also collected and identified. The color pattern and volatile profile of the floral odors are similar in the two morphs, but there are heavier compounds only identified in the floral odor extracts of glandular flowers. These compounds are linked to the presence of oil that can be perceived by bees at close distance. No difference occurs in the composition of floral visitors and no difference occurs in the reproductive success of the two morphs. Therefore, the visual and olfactory signals at long distance seem to deceive the pollinators and ensure the reproductive success also of the morph with eglandular flowers.

Key words: Floral color; floral odor; oil flowers; bees collecting oils; deceit flower.

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1 INTRODUÇÃO

Os estudos de ecologia cognitiva da polinização buscam entender como os atrativos florais (cor e odor, por exemplo) são percebidos pelos polinizadores (BRITO *et al*., 2014). No caso de espécies de plantas produtoras de óleo floral e seus polinizadores, os atrativos envolvidos na comunicação planta/polinizador ainda são pouco conhecidos (SCHÄFFLER *et al*., 2012). Neste sistema as flores produzem óleo como recurso em glândulas especializadas, que é explorado exclusivamente por abelhas coletoras de óleo (VOGEL, 1974; 1990). Como se trata de um sistema de polinização especializado, espera-se que os sinais visuais e olfativos emitidos pelas flores possuam uma relação estreita com o sistema sensorial das abelhas coletoras de óleo.

A importância dos sinais visuais e olfativos emitidos por flores de óleo na interação com seus polinizadores é conhecida, principalmente, para os sistemas de óleo do velho mundo (DÖTTERL e SCHÄFFLER, 2007; DÖTTERL *et al*., 2011; SCHÄFFLER *et al*., 2012; 2015). No entanto, a maioria das espécies de plantas produtoras de óleo ocorre em regiões neotropicais (POSSOBOM e MACHADO, 2017), o que significa que estudos focados em espécies neotropicais podem contribuir e muito para um melhor entendimento da comunicação entre flores de óleo e seus polinizadores.

Como polinizadores de flores de óleo, a maioria dos trabalhos registram as abelhas da tribo Centridini (BEZERRA, 2008; BEZERRA *et al*., 2009b; CARNEIRO *et al*., 2015; MACHADO, 2004; MELLO *et al*., 2013; RAMALHO e SILVA, 2002; VOGEL, 1974; 1990). No entanto, alguns trabalhos abordando rede de polinizadores mostram que existe tanto especialização como generalização de polinizadores (MELLO *et al*., 2013), pois até dentro de uma mesma comunidade de plantas de flores de óleo algumas espécies são polinizadas por um número maior de espécies de Centridini, enquanto outras são polinizadas por um menor número de espécies dessa tribo (BEZERRA *et al*., 2009b).

As flores de óleo apresentam variações na sua morfologia e fisiologia que podem alterar essa sinalização aos polinizadores, como a mudança de cor das flores de algumas espécies durante a antese floral, como ocorre em *Banisteriopisis muricata* e *Byrsonima gardneriana* (BEZERRA, 2008; BEZERRA *et al*., 2009a). Outro exemplo é a ausência de produção de óleo em alguns indivíduos de uma mesma espécie, como em *Banisteriopsis muricata*, *Heteropterys aceroides* e *Byrsonima sericea*, que apresentam morfos florais com glândulas de óleo (elaióforos) e morfos sem essas glândulas em uma mesma população (SAZIMA e SAZIMA 1989; TEIXEIRA e MACHADO, 2000).

Em vista disso, investigamos a interação entre flores produtoras de óleo de diferentes famílias de plantas neotropicais e seus respectivos polinizadores, com o intuito de responder perguntas direcionadas em dois artigos, relacionadas às características dos estímulos sensoriais (cor e odor). No primeiro artigo questionamos: Qual é a composição química dos odores exalados por flores produtoras de óleo? A mudança de cor das flores durante a antese é perceptível aos polinizadores? A diferença dos sinais visuais e olfativos reflete a diferença na atração dos polinizadores de flores de óleo? Para o artigo 2 procuramos entender: Em espécies que apresentam flores com e sem elaióforos (glândulas de óleo), as flores dos distintos morfos apresentam diferenças quanto à composição química de odores e/ou espectro de reflectância de cores? Como a presença desses sinais interfere na frequência dos polinizadores e no fitness reprodutivo?

1.1 PROBLEMATIZAÇÃO

1.1.1 Plantas com flores de óleo

A ocorrência de flores que produzem óleo floral em glândulas especializadas (elaióforos) e as interações entre plantas produtoras desse óleo floral e abelhas que os coletam foram apresentadas pela primeira vez em 1969 e descritas em 1974 pelo naturalista Stefan Vogel. Nesses trabalhos, Vogel afirma que essas plantas são polinizadas principalmente por abelhas fêmeas de hábito solitário, que coletam esse recurso e o misturam com o pólen para aprovisionar suas larvas (VOGEL, 1969; 1974). Na sua descoberta, Vogel ficou intrigado com as flores que possuíam o que acreditava ser na época nectários florais, mas produziam secreções oleosas (lipídeos) diferentes de néctar, cuja constituição básica são carboidratos (COCUCCI e JOHNSON, 2017). Os elaióforos, que são as glândulas produtoras de óleo, podem ser epiteliais, quando são formados por células epidérmicas secretoras, ou tricomáticos, quando são formados por placas com inúmeros tricomas glandulares que ativamente secretam óleo (VOGEL, 1974). Dr. Vogel deixou um grande legado de trabalhos abordando aspectos botânicos, entomológicos, químicos e biogeográficos envolvendo esse e outros sistemas de polinização (COCUCCI e JOHNSON, 2017; NEFF e SIMPSON, 2017).

A temática relacionada às flores de óleo progrediu e atualmente sabe-se que várias famílias de Angiospermas como, por exemplo, Krameriaceae, Malpighiaceae e Plantaginaceae, possuem espécies que produzem óleos florais como recurso para seus polinizadores (ALVES DOS SANTOS *et al.*, 2007; CARNEIRO *et al.*, 2015; MACHADO, 2004; MACHADO *et al.*, 2002; VOGEL, 1974; VOGEL e MACHADO, 1991). Ao todo, são conhecidas 11 famílias que produzem óleo como recurso para polinizadores. Para outras famílias, tais como Fabaceae, Gesneriaceae e Melastomataceae, existem indícios de espécies produtoras de óleos florais, mas esses indícios precisam ser confirmados através de estudos mais detalhados (POSSOBOM e MACHADO, 2017).

A produção de óleo surgiu diversas vezes, independentemente, em angiospermas (RENNER e SCHAEFER, 2010). A família Malpighiaceae é a mais antiga a apresentar esse recurso (RENNER e SCHAEFER, 2010) e a ausência de produção de óleo é considerada uma característica derivada na família (ANDERSON, 1979; DAVIS *et al.*, 2014; VOGEL, 1974;

1990). Algumas espécies de Malpighiaceae podem ter flores com diferentes morfos, com alguns indivíduos apresentando elaióforos e outros não, demonstrado em espécies de diferentes gêneros como *Banisteriopsis*, *Byrsonima e Heteropterys* (SAZIMA e SAZIMA, 1989; TEIXEIRA e MACHADO, 2000). Esse fenômeno é considerado uma estratégia para reduzir o alto custo enérgico envolvido na produção de óleo, sem perder o serviço da polinização prestado pelas abelhas, que visitam as flores por engano (SAZIMA e SAZIMA, 1989; TEIXEIRA e MACHADO, 2000).

Os óleos são compostos por ácidos graxos livres mono ou diglicerídeos, bastante ricos energeticamente (BUCHMANN, 1987). A composição química dos óleos é muito similar, independente da família, no entanto, estudos detalhados que identifiquem os exatos compostos de diferentes espécies ainda são escassos (POSSOBOM e MACHADO, 2017; SIMPSON *et al*., 1977;).

As famílias de flores produtoras de óleo estão presentes em diversas regiões do mundo (SCHÄFFLER *et al*., 2012, 2015) e, ao longo da região Neotropical, elas estão distribuídas em diferentes fitofisionomias (COSTA *et al*., 2006; BEZERRA *et al*., 2009; 2012; GOTTSBERGER, 1986; MACHADO *et al*., 2009; TEIXEIRA e MACHADO, 2000; VOGEL e MACHADO, 1991). Um estudo de caso realizado em fragmentos de Mata Atlântica com *Byrsonima sericea* (Malpighiaceae), mostra a relação dessa espécie com abelhas Centridini (RAMALHO e SILVA, 2002). Para 12 espécies da família Malpighiaceae, também ocorrentes em áreas de Mata Atlântica, é registrada a polinização por Centridini e Tapinotaspidini (SIGRIST e SAZIMA, 2004).

Em uma área de Caatinga, foi mostrado que *Banisteriopsis muricata* (Malpighiaceae) é a espécie de maior contribuição para a manutenção da guilda das Centridini, sendo fonte de óleo para 12 espécies de abelhas dessa tribo, destacando-se, entre as abelhas com maior número de visitas, as espécies *Centris aenea*, *C. fuscata*, *C. caxienses*, *C. flavifrons* e *C.*

tarsata (BEZERRA, 2008; BEZERRA *et al*., 2009). Outras espécies de plantas com flores de óleo encontradas na Caatinga pertencem ao gênero *Angelonia* (Plantaginaceae), que também são polinizadas por abelhas Centridini e Tapinotaspidini (MACHADO *et al*., 2002; MARTINS e ALVES-DOS-SANTOS, 2013; MARTINS *et al*., 2013; VOGEL e MACHADO 1991). Também podemos citar *Krameria tomentosa* (Krameriaceae), que ocorre em áreas abertas no nordeste do Brasil e é polinizada por abelhas Centridini (CARNEIRO *et al*., 2015).

1.1.2 Abelhas coletoras de óleo

São conhecidas até o momento cerca de 450 espécies de abelhas que coletam óleo floral (MARTINS et al., 2013). Entre essas espécies é bastante conhecida a relação das flores de óleo e as abelhas da tribo Centridini, sendo as fêmeas dessa tribo consideradas as principais polinizadoras de espécies neotropicais produtoras de óleo, havendo apenas raras exceções na tribo que não realizam coleta de óleo (ALVES DOS SANTOS *et al*., 2007; MACHADO, 2004; NEFF & SIMPSON 2017; VOGEL, 1974). Outras tribos de abelhas conhecidas por também polinizar flores de óleo são Ctenoplectini, Tapinotaspidini e Tetrapediini, todas da família Apidae (ALVES DOS SANTOS *et al*., 2007; MACHADO, 2004).

Algumas espécies de flores de óleo são auto-incompatíveis, como aquelas do gênero *Byrsonima* (SAZAN *et. al,* 2014; TEIXEIRA e MACHADO, 2000). No caso da espécie *Byrsonima cydoniifolia* frutos gerados por autofecundação são abortados (SAZAN *et. al,* 2014). Portanto espécies de flores de óleo dependem das abelhas coletoras de óleo para garantir o seu sucesso reprodutivo.

A maneira de coleta do óleo por parte das abelhas depende do tipo de elaióforo presente na espécie de planta (VOGEL 1974; MACHADO 2004; POSSOBOM e MACHADO, 2017). Algumas abelhas utilizam um conjunto de cerdas modificadas nas pernas anteriores e/ou medianas para coletar o óleo secretado pelos elaióforos, os quais posteriormente são armazenados em uma estrutura de pelos densos e ramificados (escopa) das pernas posteriores (MACHADO, 2004; ALVES DOS SANTOS *et al*., 2007). As abelhas solitárias fêmeas coletam óleo para fazer a superfície do ninho e das paredes das células, e também para alimentar as larvas, misturando óleo com pólen (ALVES DOS SANTOS *et al*., 2007; BUCHMANN, 1987). Já os machos das tribos Tetrapediini e Tapintoaspidini coletam óleo para fins ainda desconhecidos (AGUIAR e MELO, 2009; CAPPELLARI *et.* al., 2011; VOGEL e MACHADO, 1991).

1.1.3 Ecologia cognitiva em interações entre plantas de óleo e seus polinizadores especializados

As síndromes (conjunto de traços florais que pode predizer o possível polinizador) florais são definidas a partir da percepção humana dos atrativos e recursos florais, não condizendo necessariamente com a percepção dos polinizadores. As técnicas usadas nos estudos de biologia cognitiva da polinização permitem entender melhor como os grupos de polinizadores realmente são atraídos pela diversidade de flores existentes (BRITO *et al*., 2014). O estudo da biologia cognitiva de polinizadores ainda é uma área de pesquisa recente que carece de estudos que forneçam conhecimento sobre a interação entre planta e polinizador. Entre os estímulos estudados estão as cores e odores florais.

A cor das flores pode ser percebida de maneira diferente por diversos animais. As faixas visíveis de comprimentos de onda para humanos, por exemplo, estão entre 400 e 700 nm, com alguma sensibilidade acima nos 800 nm. Isso se deve a diferença dos tipos e quantidade de fotorreceptores presentes nos olhos dos animais (BRITO *et. al*, 2014). Para espécies de plantas da Austrália, as cores das flores apresentam mais frequentemente refletância entre 400 e 500 nm, que é o intervalo que os himenópteros têm maior facilidade de discriminar, mostrando assim como as cores de flores podem ter sofrido uma forte pressão por este grupo (DYER *et. al*, 2012).

Entre os insetos polinizadores, a visão da abelha *Apis mellifera* é uma das melhor estudadas e foi utilizada como um primeiro modelo de percepção de cores para abelhas como um todo(BACKHAUS, 1991). Posteriormente, um modelo foi proposto para himenópteros de maneira geral (CHITTKA, 1992). No modelo de Chittka 1992, os valores de excitação dos fotorreceptores são usados para calcular a posição ocupada por determinado estímulo que está sendo visualizado, dentro de um espaço geométrico de cores, o hexágono. Neste modelo hexagonal de cores, as distâncias são calculadas com base na visão tricomática das abelhas, UV-azul-verde (CHITTKA, 1992).

Os odores florais são capazes de atrair insetos e grupos de polinizadores (DUDAREVA e PICHERSKY, 2000) e cada espécie vegetal parece ter um cheiro único, pois o buquê floral é constituído por diversos compostos, e isso pode estar relacionado a um polinizador (ou guilda de polinizadores) em particular. As antenas dos insetos são responsáveis por captar esses odores, através dos seus sensores químicos, e cada um desses sensores possuem neurônios que se conectam diretamente com o cérebro (KAISSLING, 1986). Esses compostos químicos encontrados nos buquês florais podem ser identificados por cromatografia gasosa acoplada ao espectrômetro de massas e posteriormente testados se são percebidos pelos polinizadores da espécie de planta estudada em laboratório através da eletroantenografia (GC-EAD) e em campo através dos bioensaios (ALBUQUERQUE *et al*., 2021).

Os odores florais podem atuar tanto como atrativo quanto como recurso. Como atrativo ele pode sinalizar o tipo e/ou a disponibilidade de um determinado recurso para diversos grupos de polinizadores (VASSARIN e AMARAL-NETO, 2014). Por sua vez, como recursos, eles são coletados por machos de abelhas da tribo Euglossini (WILLIAMS e WHITTEN, 1983) e mais recentemente descoberto, por machos de abelhas coletoras de óleo

(ETL, *et al*., 2017). Em estudos realizados com espécies produtoras de óleo, foi verificada a presença do composto 1,4-diacetina no buquê de odor floral, composto esse que não é raro em outras espécies vegetais (SCHÄFFLER *et al*., 2012, 2015). Esse composto foi atrativo para a abelha coletora de óleo europeia *Macropis fulvipes* em estudos comportamentais, que gerou um comportamento de pouso e procura intensa pelas fêmeas (SCHÄFFLER *et al*., 2015). Esses autores demonstraram que quando comparadas espécies do gênero *Lysimachia*, apenas aquelas espécies que eram produtoras de óleo continham esse composto em seu buquê de odores florais, indicando assim um canal privado de comunicação entre planta e polinizador (SCHÄFFLER *et al*., 2015).

A presença da 1,4-diacetina nos odores florais de plantas produtoras de óleo parece estar ligada ao tipo de óleo produzido pela espécie vegetal, pois espécies que produzem a mesma composição de óleo (acilglicerol) produzem esse composto volátil, sugerindo que a rota metabólica da composição dos óleos florais e a do aroma floral estão relacionadas (SCHÄFFLER *et al*., 2015). Para espécies da família Malpighiaceae neotropicais foi identificada uma maior diversidade de compostos nas composições dos óleos florais (BARÔNIO *et al*., 2017), mas sua relação com o odor floral ainda não é conhecida.

A cor também está, possivelmente, relacionada com o odor floral e a rota metabólica dos compostos, para determinar esses dois atributos, parece ser a mesma (MAJETIC *et al*., 2007). Alguns grupos de polinizadores parecem ter uma preferência inata por determinado espectro de cores, como a percepção de ultravioleta (UV) pelas abelhas e a preferência por flores que refletem UV (LUNAU e MAIER, 1995).

É conhecido que algumas espécies de flores de óleo mudam o padrão de coloração de suas flores após serem polinizadas e não apresentarem mais recurso (BEZERRA, 2008). Essa estratégia de mudança de coloração pode ser entendida como uma forma de sinalização floral (KNAUER e SCHIESTL, 2015), para indicar as flores que ainda produzem recursos, e consequentemente, ainda não foram polinizadas (BEZERRA, 2008). Assim, a mudança de cor durante a antese pode interferir na atração de polinizadores (BRITO *et al.*, 2015). Por exemplo, para a espécie arbórea de floração maciça, *Tibouchina pulchra* (Melastomataceae), a manutenção das flores antigas e já polinizadas aumentam o *display* floral, tornando as plantas mais atrativas aos polinizadores a longa distância. A curta distância, ou seja, após a aproximação por parte dos polinizadores,a variação da cor (de branca para rosa sinaliza as flores que ainda possuem recursos e não foram polinizadas (BRITO *et al.*, 2015).

Dentre os representantes da família Malpighiaceae produtoras de óleo, *Banisteriopsis muricata* e *B. stellaris* apresentam uma mudança de cor nas suas flores, seguindo o evento de polinização (Bezerra 2008). *Banisteriopsis muricata* inicia a antese com flores rosa, estames amarelos, sépalas e elaióforos esverdeados, enquanto no final do dia, quando os elaióforos já estão ressecados, a coloração é alterada, as pétalas tornam-se brancas e os estames e sépalas ficam avermelhados. A variação em *B. stellaris* é mais sutil, as flores alvas no início da antese adquirem um tom pastel e vão perdendo a coloração rosa da pétala estandarte. Essa mudança de coloração possivelmente está ligada com a polinização, pois flores de *B. muricata*, que permaneceram ensacadas até o final da antese não mudam de cor (BEZERRA, 2008).

Trabalhos realizados com plantas de óleo europeias, na sua maioria, mostraram a importância dos sinais visuais e olfativos na comunicação com seus polinizadores (DÖTTERL e SCHÄFFLER, 2007; DÖTTERL *et al.*, 2011; SCHÄFFLER *et al.*, 2012, 2015). Em um estudo comparativo com plantas do gênero *Lysimachia* (Primulaceae) produtoras e não produtoras de óleo, SCHÄFFLER *et al.* (2012) mostraram que os sinais visuais e olfativos das plantas produtoras de óleo parecem estar correlacionados evolutivamente com a preferência das abelhas polinizadoras.

Como já mencionado, a síndrome floral de espécies com flores de óleo é extremamente especializada e o entendimento do papel dos sinais cognitivos visuais (cor) e olfativos (odor) precisam ser mais bem estudados. Investigar como esses sinais estão relacionados na atração dos seus polinizadores pode nos fornecer informações importantes sobre a ecologia e evolução de espécies com flores de óleo e as abelhas das tribos Centridini, Tapinotaspidini e Tetrapediini.

1.2 REFERÊNCIAS

AGUIAR, C.M.L.; ZANELLA, F.C.V.; MARTINS, C.F.; CARVALHO, C.A.L. Plantas visitadas por *Centris* spp. (Hymenoptera: Apidae) na Caatinga para obtenção de recursos florais. Neotropical Entomology 32:247-259, 2003.

AGUIAR, A.J.C., MELO, G.A.R. Notes on oil sources for the bee genus *Caenonomada* (Hymenoptera, Apidae, Tapinotaspidini). Revista Brasileira de Entomologia 53: 154–156, 2009.

ALBUQUERQUE N.S.L., MILET-PINHEIRO P., CRUZ D.D., NAVARRO D.M.A.F., MACHADO I.C. 2021. Pollination of *Sarcoglottis acaulis* (Orchidaceae) by male orchid bees: nectar as resource in spite of a strong floral scent. Plant Biology. DOI 10.1111/(ISSN)1438-8677.

ALVES-DOS-SANTOS, I.; MACHADO, I.C.; GAGLIANONE, M.C. História natural das abelhas coletoras de óleo. Oecologia Brasiliensis 11:544–557, 2007.
ANDERSON, W.R. 1979. Floral conservation in Neotropical Malpighiaceae. Biotropica 11(3):219-223.

BACKHAUS, W. 1991. Color opponent coding in the visual system of the honeybee. Vision Research, 31, 1381-1397.

BARÔNIO, G.J.; HALEEN, M.A.; MARSAIOLI, A.J.M.; TOREZAN-SILINGARDI, H.M. Characterization of Malpighiaceae flower-visitor interactions in a Brazilian savannah: How do floral resources and visitor abundance change over time. Flora 234: 128-134, 2017.

BARROS, M.A.G. Fenologia da floração, estratégias reprodutivas e polinização de espécies simpátricas do gênero *Byrsonima* Rich (Malpighiaceae). Revista Brasileira de Biologia 52:343-353, 1992.

BEZERRA, E.L.S. Guilda de flores de óleo do Parque Nacional do Catimbau: Fenologia, polinização e sistema reprodutivo. Tese de doutorado. Programa de Pós-Graduação em Biologia Vegetal, Recife, Universidade Federal de Pernambuco, 2008.

BEZERRA, E.L.S.; LOPES, A.V.; MACHADO, I.C. Biologia reprodutiva de *Byrsonima gardnerana* A. Juss. (Malpighiaceae) e interações com abelhas *Centris* (Centridini) no Nordeste do Brasil. Revista Brasileira de Botânica 32 (1): 95-108, 2009a.

BEZERRA, E.L.S.; MACHADO, I.C.; MELLO, M.A.R. Pollination networks of oil-flowers: A tiny world within the smallest of all worlds. Journal of Animal Ecology 78: 1096–1101, 2009b.

BRITO, V.; TELLES, F.; LUNAU K. Ecologia Cognitiva da Polinização. In: Rech A, Agostini K, Oliveira PE, Machado IC. (Orgs.) Biologia da Polinização. 1 ed. Rio de Janeiro: Editora Projeto Cultural, 2014.

BRITO, V.L.G.; WEYNANS, K.; SAZIMA, M.; LUNAU, K. Trees as huge flowers and flowers as oversized floral guides: the role of floral color change and retention of old flowers in *Tibouchina pulchra*. Frontiers in Plant Science 6: 362, 2015.

BUCHMANN, S.L. The ecology of oil flowers and their bees. Annual Review of Ecology and Systematics 18: 343-369, 1987.

CAPPELLARI, S.C.; MELO, G.A.R.; AGUIAR, A.J.C.; NEFF, J.L. Floral oil collection by male *Tetrapedia* bees (Hymenoptera: Apidae: Tetrapediini). Apidologie 43: 39-50, 2011.

CARNEIRO, L.T.; AGUIAR, A.J.C.; MARTINS, C.F.; MACHADO, I.C.; SANTOS, I.A. *Krameria tomentosa* oil flowers and their pollinators: bees specialized on trichome elaiophores exploit its epithelial oil glands. Flora 215: 1-8, 2015.

CHITTKA L. 1992. The color hexagon – a chromaticity diagram based on photoreceptor excitations as a generalized representation of color opponency. Journal of Comparative Physiology A: Sensory, Neural and Behavioral Physiology 170: 533–543.

COCUCCI, A.; JOHNSON, S.D. Patterns and mechanisms in plant-pollinator interactions: Stefan Vogel's contribution to contemporary pollination biology. Flora 232: 1-6, 2017.

COSTA, C.B.N.; COSTA, J.A.S.; RAMALHO, M. Biologia reprodutiva de espécies simpátricas de Malpighiaceae em dunas costeiras da Bahia, Brasil. Revista Brasileira de Botânica 29, 103-11, 2006.

DAVIS, C.C., SCHAEFER, H., XI, Z., BAUM, D.A., DONOGHUE, M.J.; HARMON, L. J. 2014. Long-term morphological stasis maintained by a plant-pollinator mutualism. Proceedings of the National Academy of Sciences 111: 5914–5919, 2014.

DÖTTERL, S.; SCHÄFFLER, I. Flower scent of floral oil-producing *Lysimachia punctata* as attractant for the oil-bee *Macropis fulvipes*. Journal of Chemical Ecology 33: 441-445, 2007.

DÖTTERL, S.; MILCHREIT, K.; SCHÄFFLER, I. Behavioural plasticity and sex differences in host finding of a specialized bee species. Journal of Comparative Physiology A 197: 1119–1126, 2011.

DUDAREVA, N. & PICHERSKY, E. Biochemical and molecular genetic aspects of floral scents. Plant Physiology 122: 627-633, 2000.

DYER, A. G.; BOYD-GERNY, S.; MCLOUGHLIN, S.; ROSA, M. G. P.; SIMONOV, V.; WONG, B. B. M. Parallel Evolution of Angiosperm Colour Signals: Common Evolutionary Pressures Linked to Hymenopteran Vision. Proceedings of the Royal Society B, 279 (1742), 3606–3615. https://doi.org/10.1098/rspb.2012.0827, 2012.

ETL, F.; FRANSCHITZ, A.; AGUIAR, A.J.C.; SCHÖNENBERGER, J.; DÖTTERL, S. A perfume-collecting male oil bee? Evidences of a novel pollination system involving *Anthurium acutifolium* (Araceae) and *Paratetrapedia chocoensis* (Apidae, Tapinotaspidini). Flora 232: 7-15, 2017.

GAGLIANONE, M.C. Interações de *Epicharis* (Apidae, Centridini) e flores de Malpighiaceae em um ecossistema de Cerrado. Anais do IV Encontro sobre Abelhas. Ribeirão Preto-SP 246-252, 2000.

GAGLIANONE, M.C. Abelhas da tribo Centridini na estação Ecológica de Jataí (Luiz Antônio, SP): composição de espécies e interações com flores de Malpighiaceae. In: Melo GAR, Alves dos Santos I, eds. Apoidea Neotropica. UNESC, 279-284, 2003.

GOTTSBERGER, G. Some pollination strategies in Neotropical Savannas and Forest. – Plant Systematics and Evolution 152: 29-45, 1986.

KAISSLING, K. E. Chemo-electrical transduction in insect olfactory receptors. Annual Review of Neurosciences 9: 121-145, 1986.

KNAUER AC, SCHIESTL FP. 2014. Bees use honest floral signals as indicators of reward when visiting flowers. *Ecology Letters* 18: 135–143.

KNUDSEN, J.T.; ERIKSSON, R.; GERSHENZON, J.; STÅHL, B. Diversity and distribution of floral scent. Botanical Review 72:1–120, 2006.

LUNAU, K.; MAIER, E.J. Innate colour preferences of flower visitors. Journal of Comparative Physiology A 177:1–19, 1995.

MACHADO, I.C. Oil-collecting bees and related plants: a review of the studies in the last twenty years and case histories of plants occurring in NE Brazil. Pp 255-280. *In:* B.M. Freitas & J.O.P. Pereira, (eds.), Solitary bees, conservation, rearing and management for pollination. Editora Impresa Universitária, UFCE, Fortaleza. 285 Pp, 2004.

MACHADO, I.C.; VOGEL, S.; LOPES, A.V. Pollination of *Angelonia cornigera* Hook. (Scrophulariaceae) by long-legged oil-collecting bees in NE Brazil. Plant Biology 4: 352-359, 2002.

MAJETIC, C.J.; RAGUSO, R.A.; TONSOR, S.J.; ASHMAN, T.L. Flower color-flower scent associations in polymorphic *Hesperis matronalis* (Brassicaceae). Phytochemistry 68: 865–874, 2007.

MARTINS, A.C.; AGUIAR, A.J.C.; ALVES DOS SANTOS, I. Interaction between oil-collecting bees and seven species of Plantaginaceae. Flora 208: 401–411, 2013.

MARTINS, A.C.; ALVES-DOS-SANTOS, I. Floral-oil-producing Plantaginaceae species: geographical distribution, pollinator rewards and interaction with oil-collecting bees. Biota Neotropica 13: 4, 2013.

MELLO, M.A.R.; BEZERRA, E.L.S.; MACHADO, I.C. Functional roles of Centridini oil bees and Malpighiaceae oil flowers in biome-wide pollination networks. Biotropica 45: 45–53, 2013.

NEFF, J.L; SIMPSON, B.B. Vogel`s great legacy: The oil flowers and oil-collecting bee syndrome. Flora 232: 104-116, 2017.

PAPADOPULOS, A.S.T.; POWELL, M.P.; PUPULIN, F. *et al*,. Convergent evolution of floral signals underlies the success of Neotropical orchids. Proceedings of the Royal Society B: Biological Sciences 280, 20130960, 2013.

POSSOBOM, C.C.F.; MACHADO, S.R. Elaiophores: their taxonomic distribution, morphology and functions. Acta Botanica Brasilica 31: 503-524, 2017.

RAMALHO, M.; SILVA, M. Flora oleífera e sua guilda de abelhas em uma comunidade de restinga tropical. Sitientibus 20: 34-43, 2002.

REIS, M.G.; FARIA, A.D.; ALVES-DOS-SANTOS, I.; AMARAL, M.C.E.; MARSAIOLI, A.J. Byrsonic acid – the clue to floral mimicry involving oil-producing flowers and oil-collecting bees. Journal of Chemical Ecology 33: 1421-1429, 2007.

RENNER, S.S.; SCHAEFER, H. The evolution and loss of oil-offering flowers: new insights from dated phylogenies for plants and bees. Philosophical Transactions of the Royal Society B 365: 423-435, 2010.

SAZAN, M.S.; BEZERRA, A.; DIEGO. M.; FREITAS, B.M. 2014. Oil collecting bees and *Byrsonima cydoniifolia* A. Juss. (Malpighiaceae) interactions: the prevalence of long-distance cross pollination driving reproductive success. Anais da Academia Brasileira de Ciencias 86: 347-357.

SAZIMA, M.; SAZIMA, I. Oil-gathering bees visit flowers of eglandular morphs of the oil-producing Malpighiaceae. Botanica Acta 102: 106-111, 1989.

SCHÄFFLER, I.; BALAO, F.; DÖTTERL, S. Floral and vegetative cues in oil-secreting and non-oil-secreting *Lysimachia* species. Annals of Botany 110: 125-138, 2012.

SCHÄFFLER, I.; STEINER, K.E.; HAID, M.; VAN BERKEL, S.S.; GERLACH, G.; JOHNSON, S.D.; WESSJOHANN, L.; DÖTTERL.; S. Diacetin, a reliable cue and private communication channel in a specialized pollination system. Scientific Reports 5: 12779, 2015.

SCHIESTL, F.P.; POLL, F.M. Detection of physiologically active flower volatiles using gas chromatography coupled with electroantennography. In Molecular Methods of Plant

Analysis, Volume 21: Analysis of Taste and Aroma, JF. Jackson HF. Linskens and R. Inman, eds. Berlin, Germany: Springer, pp. 173–198, 2002.

SIGRIST, M.R.; SAZIMA, M. Pollination and reproductive biology of twelve species of neotropical Malpighiaceae: stigma morphology and its implications for the breeding system. Annals of Botany 94: 33–41, 2004.

SIMPSON, B.B., NEFF, J.L., SEIGLER, D. *Krameria*, free fatty acids and oil-collecting bees. Nature 267: 150–151, 1977.

TEIXEIRA, L.M.; MACHADO, I.C. Sistemas de polinização e reprodução de *Byrsonima sericea* DC (Malpighiaceae). Acta Botanica Brasilica 14: 347-357, 2000.

VARASSIN, I.G.; AMARAL-NETO, L.P. Atrativos. In: Rech A, Agostini K, Oliveira PE, Machado IC. (Orgs.) Biologia da Polinização. 1 ed. Rio de Janeiro: Editora Projeto Cultural, 2014.

VOGEL, S. Flowers offering fatty oil instead of nectar. *In:* XI Proc. Int. Bot. Cong. Abstracts. Seattle. 229, 1969.

VOGEL, S. Ölblumen und ölsammelnde Bienen. Tropische und Subtropische Pflanzenwelt 7: 285-547, 1974.

VOGEL, S. History of the Malpighiaceae in the light of pollination ecology. Memoirs of the New York Botanical Garden 55: 130-142, 1990.

VOGEL, S.; MACHADO, I.C. Pollination of four sympatric species of *Angelonia* (Scrophulariaceae) by oil collecting bees in NE Brazil. Plant Systematics and Evolution 178: 153-178, 1991.

WILLIAMS, N.H.; WHITTEN, W.M. Orchid floral fragrances and male Euglossine bees: methods and advances in the last sesquidecade. Biological Bulletin 164: 355-395, 1983.

2 ARTIGO 1 - Visual and olfactory cues of some Neotropical oil flowers: spectrophotometric and chemical characterization

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- **Background and Aims** Visual and olfactory floral cues play a pivotal role in plant-pollinator communication. These cues are normally attractive to pollinators and may signal the presence of a particular resource, and, in many cases, color changes allow the visitor to differentiate pollinated from unpollinated flowers. In some angiosperm families, flowers produce oil as reward for pollinators. The floral oils are collected by females of some bee tribes, which use the oil for provisioning offspring and to build nests. In these specialized associations, the floral signals involved in plant-pollinator interaction remain largely unknown. In this work, we provide more knowledge about the color and/or scent of some Neotropical oil flowers.
- **Methods** Color of 11 species of four different families, Malpighiaceae, Plantaginaceae, Krameriaceae and Iridaceae, were characterized using spectrophotometry and interpreted under the bee vision model. Scent samples from flowers and leaves of 12 species of Malpighiaceae, one of Krameriaceae and one of Plantaginaceae were extracted using *n*-hexane and analyzed by gas chromatography coupled to mass spectrometry.
- **Results** The color signals of the species studied appear to show diverse patterns, and the color change of the flowers of some species helps the pollinators to identify flowers in

different stages of anthesis. The same tendency was observed for scents: some species produce a floral scent composed of a high number of compounds, most of which still unknown, with a strong interspecific variation. Pentacosane was the most common compound found in the flowers (reported in 8 species).

- **Conclusions** Visual and olfactory cues are divergent in the species in our study, showing that they are species-specific traits. This diversity of compounds in the bouquet of oil flowers may be linked to the difference in the richness and diversity of oil collecting bees that visit different oil flowers.

Key words: Floral color, floral scent, floral signaling, oil bee, resources/attractive, Malpighiaceae, Krameriaceae, Iridaceae, Plantaginaceae.

2. 1 INTRODUCTION

The characteristic of producing floral oil as a reward for pollinators arose independently in angiosperms several times (Possobom and Machado, 2017; Renner and Schaefer, 2010). There is a great diversity of Neotropical oil-producing species, distributed in many families, such as Malpighiaceae, Plantaginaceae, Orchidaceae, Iridaceae, Cucurbitaceae, Solanaceae, Primulaceae, and Krameriaceae (Vogel, 1969; Machado, 2004; Alves dos Santos *et al*., 2007). The Malpighiaceae, for example, have a great number of oil-producing species, about 1,260 species distributed in 75 genera (Zappi, 2015).

It is known that the pollination of oil flowers is performed mainly by female bees of solitary species of the tribe Centridini. The females collect this resource to mix with pollen providing it to their offsprings. The oils are also used for the construction of nests (Vogel, 1974; Alves dos Santos *et al*., 2007). Female bees of other tribes, such as Ctenoplectrini, Tapinotaspidini and Tetrapediini (Apidae), also pollinate oil flowers, but they are not as diverse as Centridini bees (Machado 2004; Alves dos Santos *et al*., 2007).

Studies investigating the communities and network of oil-bees and oil flowers in the Caatinga, a Tropical Dry Forest in the northeast of Brazil, have shown that some species are visited and pollinated by a small number of oil bee species, whereas others by a larger number of oil bees (Bezerra *et al*., 2009b; Mello *et al*., 2013; Martins and Alves-dos-Santos, 2013). Furthermore, some oil bee species are more generalists, whereas other are more specialists in terms of host plants used as oil source. In this sense, it is possible that visual and olfactory floral cues are related to these differences in the spectrum of pollinators, modeling the interactions between oil flowers and oil-collecting bees.

The importance of olfactory cues produced in oil flowers have been the subject of few studies, which revealed the importance of these cues in plant-pollinator communication (Dötterl and Schäffler, 2007; Dötterl *et al*., 2011; Schäffler *et al*., 2012; Schäffler *et al*., 2015). So far, we know that some species have a compound, 1.4 diacetin, which is present in the floral scent bouquet of oil-flower species. This compound is expected in plants that have oils with a glycerol moiety and an acetyl group, and acts in attracting oil-collecting bees (Schäffler *et al*., 2015).

For the color trait, a similar color pattern (UV-green/Green) was found for *Lysimachia* species that produce oil flowers, which was not shared with congeneric non-oil species (Schäffler *et al*., 2012). Color changing of flowers is the subject of investigation in some works (Bezerra, 2008; Bezerra *et al*, 2009a; Melo *et al*., 2018), and some species of oil flowers change the color pattern of their flowers after being pollinated (Bezerra, 2008; Bezerra *et al*, 2009a). In the case of *Byrsonima variabilis* (Malpighiaceae), the color change of the flag petal is related to the amount of pollen (Melo *et al*., 2018).

Therefore, we still don’t understand how communication and signaling occurs between these Neotropical floral oil species and their pollinators (Schäffler *et al*., 2012). Consequently, more research is need if we want to understand in more details the communication between oil flowers and their pollinators. So, in this study we investigated the visual and olfactory floral cues of Neotropical oil plants, addressing the following questions: 1) What is the color pattern of the flowers of the studied species? 2) How colour changes of oil flowers are perceived by bees? 3) What is the chemical composition of the scent exhaled by oil flowers? 4) Is the floral signaling of oil flowers plants related to pollinator specificity?

2.2 MATERIALS AND METHODS

2.2. 1 Study species and sites

The visual cues were measured and analyzed from 11 species of oil flowers, belonging to the families Malpighiaceae (N = 7), Plantaginaceae (N = 2), Krameriaceae (N = 1) and Iridaceae (N = 1) (Table 1; Fig.1). Scent samples were collected from flowers of 14 oil-producing species, belonging to the families Malpighiaceae (N = 12), Plantaginaceae (N = 1) and Krameriaceae (N = 1) (Table 1). Also, a survey on the species of bees that visit the studied oil flowers was performed in the specialized literature (Bezerra *et al*. 2009; Carneiro *et al*., 2015; Melo *et al*., 2013; Santos *et al*., 2016; Teixeira and Machado, 2000; Vogel 1974; Vogel and Machado 1991) (Table 1).

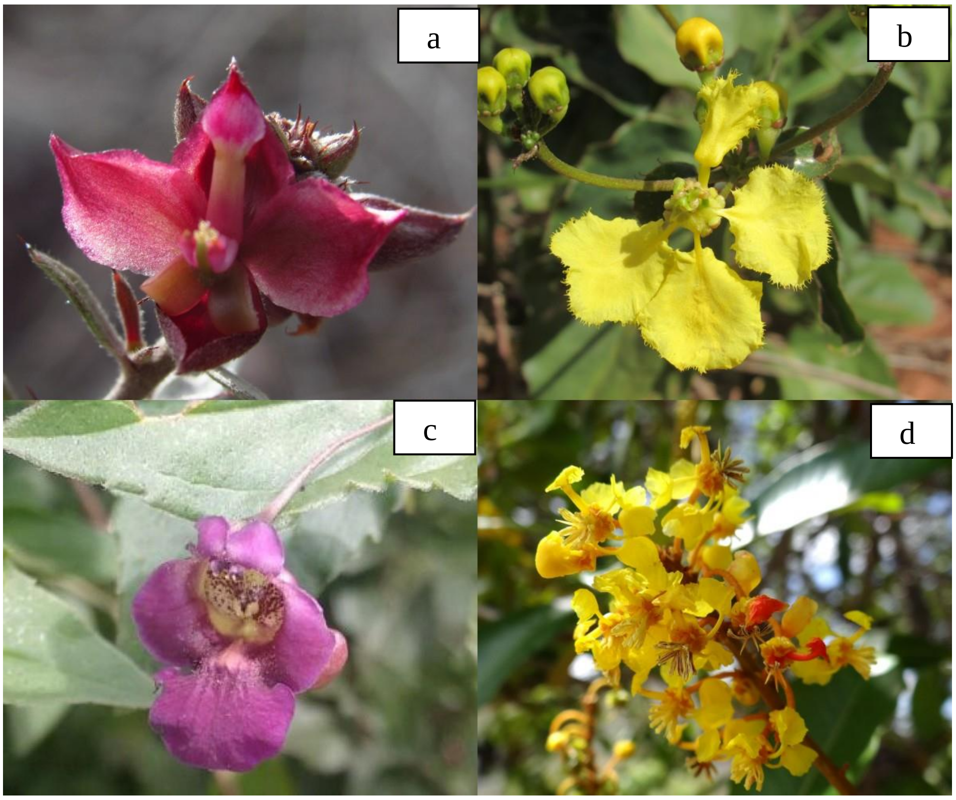


Figure1. Flowers of the investigated oil plants. (a) *Krameria tomentosa* (Krameriaceae), (b) *Stigmaphyllon auriculatum* (Malpighiaceae) (c) *Angelonia campestris* (Plantaginaceae) and (d) *Byrsonima sericea* (Malpighiaceae). Fonte: Autor, 2021.

TABLE 1. List oil plants studied, region of collection, sample sizes of color and scent analyzed and a list of oil-bee species visiting the plants investigated here according to the literature. Missing information = -.

Family/Species	Floral visitors	References (Floral visitors)	Collection Region	Number of samples Floral color	Number of samples Scent: flower/ vegetative
Malpighiaceae					
Camerea axillares			Campo Rupestre (Caetité)	-	4/2
Banisteriopsis muricata	*Centris aenea, Centris fuscata, Centris caxienses, Centris tarsata, Centris flavifrons, Centris trigonoides, Apis mellifera, Epicharis* sp2*, Xylocopa sp, Centris obsoleta, Centris* sp.1*, Xylocopa grisescens*	Bezerra et al., 2009	Caatinga (Catimbau)	5	-
Banisteriopsis stellaris	*Centris aenea, Centris fuscata, Centris tarsata, Centris caxienses*	Bezerra et al., 2009	Caatinga (Catimbau)	-	5/1
Banisteriopsis gardneriana	-		Campo Rupestre (Licínio de Almeida)	-	5/1
Byrsonima sericea	*Centris aenea, C. analis, C. flavifrons, C. frontalis, C. fuscata, C. leprieuri, C. maculata, C. minuta, C. ptilopoda, C. spilopoda, C. varia, Epicharis bicolor, E. fasciata, E. flava, E. ligulata, E. maculata, Epicharis* sp.*, Paratetrapedia* sp.*, Trigona fulviventris, Augochloropsis* ap.	Teixeira, Machado, 2000	Floresta Atlântica (Aldeia and UFPB)	5	10/2
Byrsonima crassifólia	*Centris aenea, C. tarsata*	Mello et al., 2013	Floresta Atlântica (Guaribas)	-	4/1

Byrsonima gardneriana	*Centris aenea, C. fuscata, C. caxienses, C. tarsata, C. flavifrons, Apis milifera*	Bezerra et al., 2009	Caatinga (Catimbau)	5	7/1
Diplopterys pubipetala	*Centris aenea, Centris fuscata, Centris tarsata, Centris flavifrons, Centris caxienses*	Bezerra et al., 2009	Caatinga (Catimbau)	-	5/1
Galphimia brasiliensis	-		Floresta Atlântica (UFPB)	5	5/1
Heteropterys sp.	-		Caatinga (Catimbau)	5	-
Heteropterys byrsonimifolia	-		Campo Rupestre (Caetité)	-	5/2
Stigmaphyllon paralias	*Centris aenea, C. fuscata*	Bezerra et al., 2009	Caatinga (Catimbau) e Floresta Atlântica (Sairé e Guaribas)	5	7/2
Stigmaphyllon auriculatum	*Centris aenea, C. fuscata, C. caxienses, C. flavifrons*	Bezerra et al., 2009	Caatinga (Catimbau)	5	5/2
Krameriaceae					

Krameria tomentosa	*Centris nitens, C. tarsata, C. hypditis, Arhysoceble huberi, Trigona fulviventris*	Carneiro et al., 2015	Floresta Atlântica (Guaribas)	5	10/1
Plantaginaceae					
Angelonia campestris (sin. *Angelonia hookeriana)*	*Centris* (*Paracentris*) *xanthomelaena, Tetrapedia* cf *rugulosa, Plebeia mosquito, Trigona spinipes*	Vogel; Machado 1991	Floresta Atlântica (Sairé)	5	-
Angelonia biflora	*Centris* (*Hemisiella*) *trigonoides, C.* (*Trachina*) *fuscata*	Vogel 1974	Floresta Atlântica (Sairé)	5	5/2
Iridaceae					
Cipura paludosa	*Plebeia sp*., *Augochlora thalia*	Santos et al., 2016	Floresta Atlântica (Sairé)	5	-

Fonte: Autor, 2021.

The fieldworks were conducted in populations from different ecosystems: Two dry forest (Caatinga, one area, Campo Rupestres, two areas) and a Rain Forests (Atlantic forest, four areas) in NE-Brazil. In the Caatinga, studies were conducted in the Catimbau National Park (8 ° 30 '57 "S 37 ° 20' 59" O), with altitudes ranging from 600 m to 1000 m (Rodal *et al*., 1998); the climate with temperature annual average is 26 ° C (SUDENE, 1990) and the average annual rainfall is 358 mm (CLIMATE-DATE-ORG, 2019). In Campo Rupestre the studies sites were in the city of Caetité and Licínio de Almeida in the state of Bahia. Caetité has an average temperature of 22.0 °C and the average annual rainfall is 885 mm. The average annual temperature in Licínio de Almeida is 22.4 °C, and 597 mm is the average annual rainfall (CLIMATE-DATE-ORG, 2019). In the Atlantic Forest, studies were carried out in four different fragments. We collected and analysed flowers in the Guaribas Biological Reserve (7º6´S, 34º52´ W), which has temperature average annual is 25ºC, with a total annual rainfall is 1875 mm (Brazil, 2003). The fragments of “Aldeia” (7º56'14"S, 35º01'57"W), and of Campus I of the Federal University of Paraíba (7º08'16'S, 34º51'19"W) have a warm and humid climate, with an annual average temperature close to 25ºC and total precipitation ranging between 1,500 and 1,700 mm (CLIMATE-DATE-ORG, 2019; Lima and Heckendorff, 1985). In Sairé (8 ° 19 ′ 42 ″ S, 35 ° 41 ′ 23 ″ O), the annual average temperature is 21 ºC, with 842 mm of average annual rainfall.

2.2. 2 Color measurements of oil flowers and bee color hexagon

The color reflectance of the flowers was measured with a spectrophotometer (JAZEL200, Ocean Optics, USA) (Campbell *et al*., 2012). For each species, five flowers of different individuals, one per individual, as well as their young leaves, three per individual (leaves next to flowers), were measured *in situ*. Measurements were made from the petals, sepals and tepals, with emphasis on the variation of reflectance observed in the flag petals of Malpighiaceae, the lip of *Angelonia* (Plantaginaceae), upper sepals of Krameriaceae and external tepals of *Cipura paludosa* (Iridaceae). For *Banisteriopsis muricata* and *Byrsonima gardneriana* (Fig. 2), we measured the flag petals, the stamens and the stigmas at different times of the anthesis (five first-day and five second-day flowers). Anthesis data were taken from the literature (Bezerra, 2008). The stamens and stigmas were measured only for these two species for greater detail of color changes.

The mean reflections of each floral part and leaves were used to determine the colour loci of each structure in the hexagon colour space (Chittka and Kaven, 2005). The reflectance of leaves was used as color background of their respective species. The hexagon model of vision of the bees is subjectively divided into six parts: UV, UV-blue, blue, blue-green, green and UV-green (Chittka and Kaven, 2005). Color distances around 0.05 hexagon units in the hexagon are poorly discriminated, whereas distances > 0.10 are easily discriminated by the bees (Dyer and Chittka, 2004).

Figure 2 - Flowers of the investigated change colour oil plants (a) *Banisteriopsis muricata* (first-day flowers), (b) *Banisteriopsis muricata* (second-day flowers) and (c) *Byrsonima gardneriana* (first-day flowers) (Malpighiaceae). Fonte: Autor, 2021.

2.2.3 Collection and analysis of floral scent

In order to characterize the floral scent chemistry of the investigated species, floral scent samples were collected in the morning. For each sample, five flowers were excised from the pedicel using a scissor, and inserted into 2 ml vials ("Analitica") filled with 1 ml of solvent (hexane) for 2 min. The samples were filtered with glass wool and concentrated to 0.5 ml with nitrogen gas flow. The same protocol was performed for the leaves. Three young leaves (leaves next to flowers) were used to constitute one sample for each species.

The solvent samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS; Agilent 7890A™ gas chromatograph, Agilent 5975C SeriesMSD™ mass spectrometer, Palo Alto, USA), equipped with a non-polar HP-5ms™ column (Agilent J&W; 30 m x 0.25 mm d.i.,0.25µm film thickness) and a thermal separation probe (TSP, Agilent, Palo Alto, USA). Aliquots of the solvent samples (1µL) were placed in a microvial, which was loaded into the probe and inserted into the modified GC injector. The GC injector worked at a temperature of 250 ºC. GC oven temperature was set at 40 ºC for 1 min, then increased at a rate of 6ºC min^{-1} to 290ºC, and compounds were identified through GCMS Solution Version 2.72 (1999-2012).

Compounds were identified by comparing to those mass spectra and retention indices with that authentic reference sample available from commercially available mass spectral libraries (Adams, Essential Oils 23P, FFNSC 2, STANDARDSSA, W9N11). The relative amount of each compound is measured by the area of the curves in the chromatograms. The compounds that were also found in the leaf were excluded from the list of floral scent compounds.

The chemical composition of floral scents (relative amounts of compounds) of the investigated species was compared through an analysis of similarity (ANOSIM), using a *Bray-Curtis* dissimilarity matrix, followed by *a posteriori* pairwise comparisons. In order to visually depict the difference/similarity in floral scent bouquet of the species, a non multidimensional Scaling (NMDS) analyses was performed. The *S. paralias* samples were removed in NMDS as they did not contain any compounds in common with the other species. The family and genera of each species was also analyzed separately for comparison. All analyses were performed in the software Primer 6 (Clarke and Gorley, 2006).

2.3 RESULTS

2.3.1 Leaf and floral colour properties

The leaves of all investigated species had similar reflectance properties, with peaks around 550 nm (Fig. S1a). The flag petals of Malpighiaceae, the lip of *Angelonia* (Plantaginaceae), upper sepals of Krameriaceae and external tepals of *Cipura paludosa* (Iridaceae) of flowers of the analyzed species diverged in the peaks of reflectance, and also diverged when plotted on the hexagon vision model of bees (Fig. 3; Fig S1b).

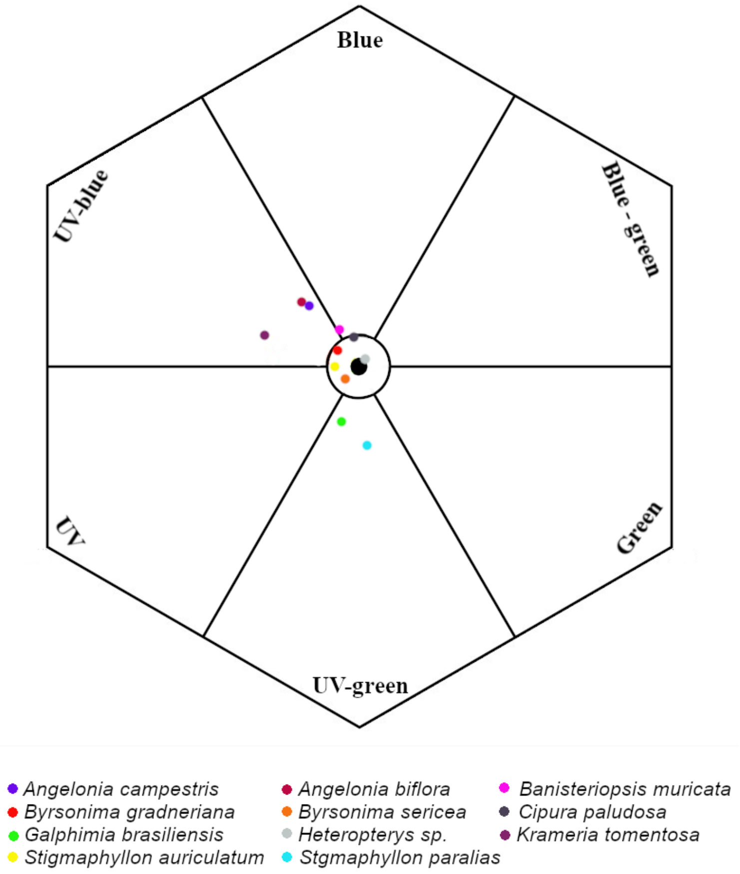


Figure 3 - Petal colours of eleven floral oil species of the families Plantaginaceae, Malpighiaceae, Krameriaceae and Iridaceae, displayed in the hexagon color space. Fonte: Autor, 2021.

In the bee hexagon model, the species *Angelonia biflora* and *A. campestris*, *Krameria tomentosa*, *Banisteriopsis muricata*, *Byrsonima gardneriana* and *Stigmaphyllon auriculatum* have UV-blue coloring. The colour locus of the flowers of *Cipura paludosa* (Iridaceae) falls within the bee-blue category, whereas that of *Byrsonima sericea* within the bee-UV category. The petals of *Galphimia brasiliensis*, which has a reduction in its elaiophores, have a UV-green color, like *Stigmaphyllon paralias*, another Malpighiaceae (Fig.3).

The color of the petals of *A. biflora*, *A. campestris*, *K. tomentosa*, *B. muricata*, *G. brasiliensis* and *S. paralias* are easily discriminated by bees (distance of color loci to the achromatic center > 0.1 hexagon units) whereas the color of the petals of *B. gardneriana*, *B. sericea*, *C. paludosa*, *S. auriculatum* and *Heteropterys* sp. are indistinguishable or hard to distinguish (distance of color loci to the achromatic center < 0.1 hexagon units) (Table 2).

TABLE 2. Distances (in hexagon units) between flower color (flag petal) of 11 oil flower species of different families (values above 0.1 hexagon units are in bold) and distances of each flower color to the hexagons center (in hexagons units). Ab = *Angelonia biflora*, Ac =*Angelonia campestris*, Byg = *Byrsonima gardneriana*, Bm = *Banisteriopsis muricata*, Bys = *Byrsonima sericea*, Cp= *Cipura paludosa*, Gb = *Galphimia brasiliensis*, Kt = *Krameria tomentosa*, H = *Heteropterys* sp., Sa = *Stigmaphyllon auriculatum* and Sp = *Stigmaphyllon paralias.*

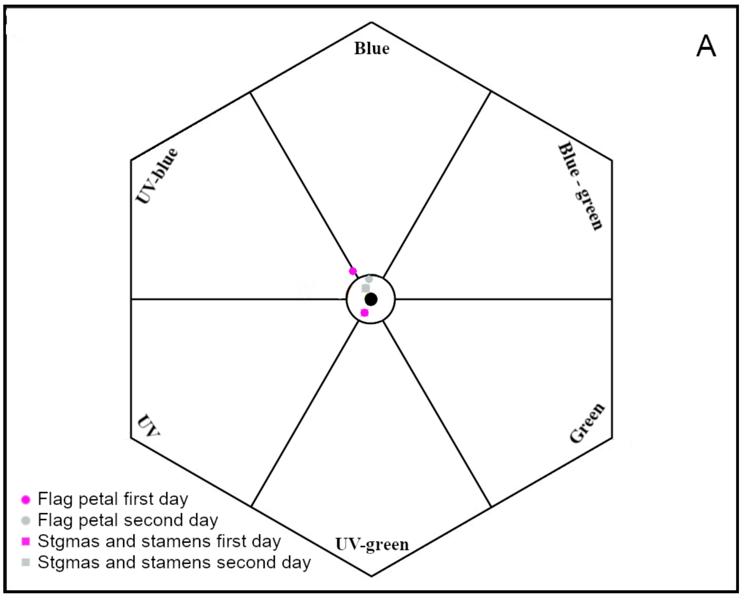
	Ab	Ac	Byg	Bm	Bys	Cp	Gb	Kt	H	Sa	Distance to the center
Angelonia biflora											**0.241**
Angelonia campestris	0.020										**0.222**
Byrsonima gardneriana	**0.167**	**0.149**									0.076
Banisteriopsis muricata	**0.129**	0.057	**0.108**								**0.118**
Byrsonima sericea	**0.246**	0.083	**0.230**	**0.139**							0.053
Cipura paludosa	**0.173**	0.057	**0.153**	0.045	**0.120**						0.084
Galphimia brasiliensis	**0.352**	**0.201**	**0.338**	**0.258**	**0.120**	**0.240**					**0.163**
Krameria tomentosa	**0.138**	**0.209**	**0.147**	**0.208**	**0.257**	**0.248**	**0.324**				**0.280**
Heteropterys sp	**0.238**	0.081	**0.218**	**0.110**	0.081	0.070	**0.189**	**0.290**			0.027
Stigmaphyllon auriculatum	**0.203**	0.047	**0.187**	**0.105**	0.044	0.098	**0.154**	**0.216**	0.089		0.068
Stigmaphyllon paralias	**0.439**	**0.278**	**0.424**	**0.332**	**0.194**	**0.305**	0.098	**0.422**	**0.241**	**0.236**	**0.220**

Fonte: Autor, 2021.

2.3.2 Changes in flower color properties

Some species of Malpighiaceae change their colors during the anthesis, such as *Banisteriopsis muricata* and *Byrsonima gardneriana*. The flag petals of *B. muricata* have two peaks of reflectance at the wavelengths between 400 - 500 nm and between 600 - 700 nm on the first–day of anthesis and one peak at 400 - 700 nm on the second-day of anthesis (Fig. S2a). The flag petals of *Byrsonima gardneriana* have peaks of reflectance at 400 - 700 nm and change to 600 - 700 nm (Fig. S2b). The color of the flag petals of flowers of first and second days of *Banisteriopsis muricata* was plotted within the UV-blue and blue bee color, respectively. Also, both the stamens and stigmas of first-day flowers plotted in the UV-green field, whereas the stamens and stigmas of second-day flowers plotted in the UV-blue and blue bee color, respectively (Fig.4a). The color of flag petals of *Byrsonima gardneriana* of first day is within the achromatic center and second days is within the UV-blue bee colour, and the stamens and stigmas of first-day flowers fall within the blue/green bee color, whereas both the stamens and stigmas of second-day fall within the UV-blue category (Fig. 4b).

The color of the flag petals of first-day flowers of *Banisteriopsis muricata* are easily distinguishable from the background by bees (0.12 hexagons units away from the center), but this was not the case of the flag petals of the second-day and stamens and stigmas of first and second-day (< 0.1 hexagons units). For the *Byrsonima gardneriana*, the flag petals of first-day flowers are not so easily visible (0.07 hexagons units away from the center), but there is a difference between the structures of the first and second-day flowers (0.38 between stamens and stigmas and 0.13 between flag petals of the first and second days) (Table 3).



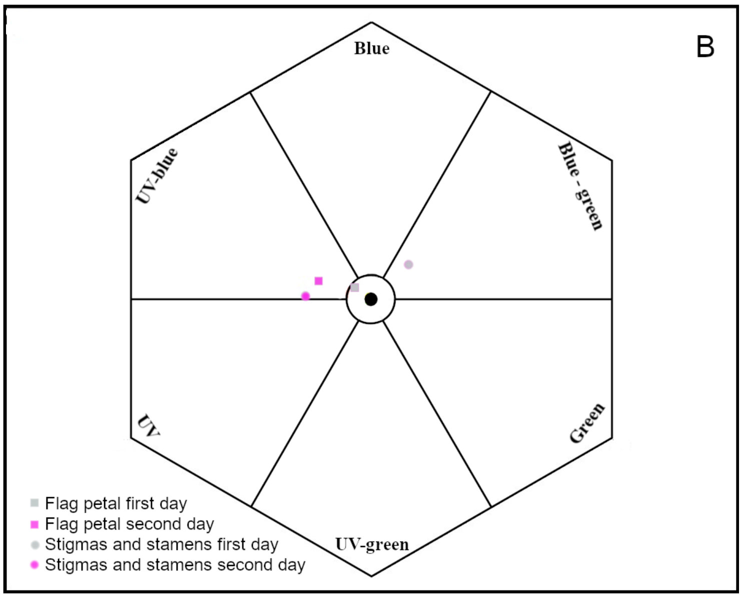


Figure 4. Petal colours of (A) *Banisteriopsis muricata* and (B) *Byrsonima gardneriana* (Malpighiaceae) displayed in the bee color hexagon. Fonte: Autor, 2021.

TABLE 3. Distances (in hexagon units) between flower color of *Banisteriopsis muricata* and *Byrsonima gardneriana* (Malpighiaceae) (values above 0.1 units are in bold) and distances of each flower color to the acromatic center. FP = flag petal; and S = stigmas and stamens

	First – day flowers		*Second – day flowers*	
	S	FP	S	Distance to the center
Banisteriopsis muricata				
First – day				
S				0.05
FP	**0.15**			**0.12**
Second – day				
S	0.09	0.07		0.04
FP	**0.12**	0.06	0.03	0.07
Byrsonima gardneriana				
First – day				
S				**0.18**
FP	**0.20**			0.07
Second – day				
S	**0.38**	**0.18**		**0.24**
FP	**0.32**	**0.13**	0.06	**0.20**

Fonte: Autor, 2021.

2.3.3 Qualitative properties in floral scents

In the scent samples, we found a total of 288 compounds in the flowers of the 14 species. From the compounds, 67 were identified (Table S1). The scent bouquet of *Krameria tomentosa* was the most complex with 57 compounds, followed by *Byrsonima sericea* (49 compounds), *Angelonia biflora* (45 compounds), *Byrsonima crassifolia* (40 compounds), *Galphimia brasiliensis* (33 compounds), *Banisteriopsis muricata* (31 compounds), *Banisteriopsis gardneriana* (25 compounds), *Banisteriopsis stelaris* (22 compounds) *Stigmaphyllon auriculatum* (21 compounds), *Diplopterys pubipetala* (18 compounds), *Camerea axillares* (17 compounds) *Heteropterys byrsonimifolia* (13 compounds), *Byrsonima gardneriana* (10 compounds) and *Stigmaphyllon* *paralias* (10 compounds). Although many compounds are shared by several species, there is not a single one that is ubiquitous in all sampled species. Pentacosane was the most common compound found in the flowers (reported in 8 species), followed by Hexadecanoic acid 1-methylethyl ester, Heptacosane and Hentriacontane (7 species).

The ANOSIM analyses revealed that the chemical composition of the floral scent bouquets (relative amount of compounds) differ significantly among species (ANOSIM, r = 0.94; p = 0.001) and a posteriori pairwise comparisons indicated that each species has its own scent profile (see Table S2 for a statistical output of pairwise comparisons; Fig. 5). Furthermore, we found a significant difference between the scent chemistry of different genera (ANOSIM, r = 0.53; p = 0.001) (Table S3), but not of different families (ANOSIM, r = 0.068; p = 0.16).

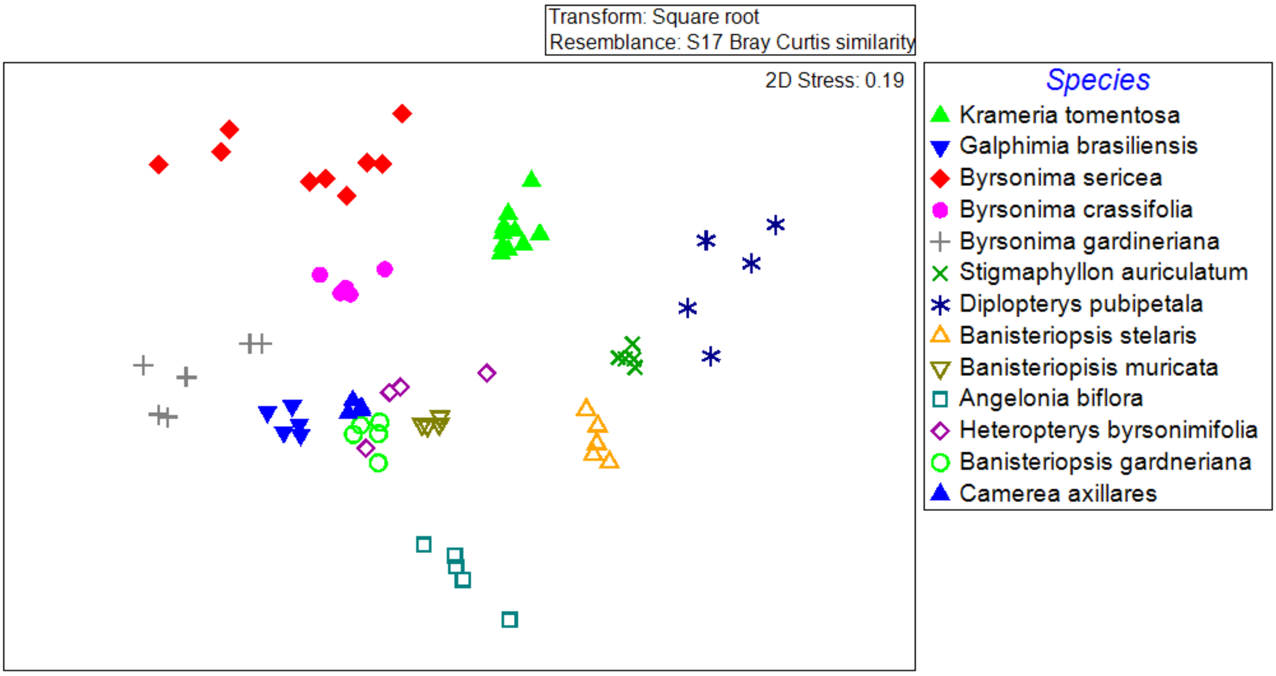


Figure 5 - Multidimensional Scaling (MDS) of samples of floral scents of 13 floral-oil species from two plant families (Krameriaceae, Plantaginaceae and Malpighiaceae). Fonte: Autor, 2021.

2.4 DISCUSSION

In the present study, we investigated the floral color and floral scent of some Neotropical oil plants, and we found a great diversity in these cues among the studied species. Most works carried out with European species highlighted the importance of these signals in plant-pollinator communication and showed a private communication channel mediated by the volatile compound 1,4-Diacetin (Dötterl and Schäffler, 2007; Dötterl *et al.*, 2011; Schäffler *et al.*, 2012, 2015), which was not found in the neotropical species studied. In plant species of the genera *Lysimachia* (Primulaceae), the oil-producing and non-oil-producing flowers were compared, showing that the visual and olfactory of oil flowers cues seem to be evolutionarily correlated with the preference of pollinating bees (Schäffler *et al.*, 2012). The oil diversity of Neotropical species may explain the diversity of floral compounds found in oil flowers, is known that the metabolic route of floral oil composition and that of floral aroma are related (Schäffler *et al.*, 2015).

2.4.1 Floral color properties

The floral-oil plants investigated in this study showed a diversity of colors in their petals, thus there is no single color pattern to indicate the production of the oil resource for the oil-collecting bees. In specialized systems, when the species depend on the same pollinator to reproduce, they have driven the convergent evolution of floral signals, in which similar floral traits appeared in distantly related taxa (Rodríguez-Gironés and Santamaría, 2004). This happens with food-deceptive orchids that share the oil bee pollinators with Malpighiaceae species, and present similar floral color, which are probably indistinguishable by the pollinators (Papadopulos *et al.*, 2013). For species of the genus *Lysimachia* (Primulaceae), oil species of the genus present a bee-green pattern, while non-oil species of the genus have the most varied color pattern (Schäffler *et al.*, 2012). In the European systems, there is not a great diversity of oil bees (Schäffler *et al.*, 2012), so the selective pressure on the visual signals is exerted by one or a few bee species, such that the color patterns are very clear. The same process seems to occur with the floral signals of species of the same genus *Campanula* (Campanulaceae) (Milet-Pinheiro *et al*., 2021). In Neotropical systems we know nothing about innate color preferences of oil bees. It could be that there is a large discrepancy in this preference, such that colors are subject to divergent selective pressures according to the different pollinator species that visit Neotropical oil flowers.

2.4.2 Change in floral color along the anthesis

The flag petals of first-day flowers of *Banisteriopsis muricata* are visible, as they are not in the achromatic center of vision of the bees (0.12 units away from the center). This color change is possibly linked to pollination, since flowers of *B. muricata* that remained bagged until the end of the anthesis did not change the color (Bezerra, 2008, Bezerra *et al*., 2009a). Therefore, the color of the standard petal at the beginning of anthesis is an indicator that the plant still has the resource, in this case oil, for the pollinator, the oil collecting bees. The presence of nectar guides, for example, is a case of visual signaling of the resource (Proctor *et al*., 1996), which has already been shown that its absence can even decrease the female and male fitness of a plant (Hansen *et al*., 2011). In manipulation experiments, its removal had little effect on the pollinator's approaches, but it did decrease the proboscis insertion (Hansen *et al*., 2011).

In *Byrsonima gardneriana* the set of differences between the structures seems to be a factor that can indicate to the pollinators the flowers of the first-day, which still have resources (oil) and have not yet been pollinated. This suggests a case of honest signaling to pollinators, which might on the one hand optimize foraging efficiency of pollinators by indicating flowers that still have resource (Knauer and Schiestl, 2015) and, on the second hand, increase the chance of pollen transfer to flowers that are still not pollinated (Brito *et al*., 2015). The color change during anthesis can interfere with the attraction of pollinators (Brito *et al*., 2015). In *Tibouchina pulchra* (Melastomataceae), for example, the maintenance of old flowers increases the floral display making the plants more attractive to pollinators over a long distance. At short distances, the color variation within the crown indicates the flowers that still have resources and have not yet been pollinated (Brito *et al*., 2015). Pollinators learn to associate resource availability with a particular color, and this is advantageous both for the plant, which will guide pollinators to flowers not yet pollinated, and for the pollinators, which will have a higher foraging efficiency. This associative learning is already known for bees (Menzel, 1993).

The classification of the floral characteristics according to human perception is no longer indicated, as we need to understand how pollinators species recognize these floral signals (Ollerton *et al*, 2009). In this scenario, a new area of the study of pollination biology emerges, the cognitive biology of pollination, which aims to understand the perceptions of pollinators about these floral signals (Brito *et al*., 2014). Curiously, the differences between flag petals of first- and second-day flowers of *Banisteriopsis muricata* is quite subtle under the bee color hexagon (0.06 hexagons units), a result that is in contrast with the evident color difference as perceived by the human eyes (pink and white, respectively). This shows the importance of investigating the floral cues from the pollinator perspective because what seems to be different for us humans may not be for the bees.

2.4.3 Floral scents of floral-oil species

The Neotropical oil-producing species in our work showed a large number of compounds in floral scent, most of which are apparently unknown, so identification based on GC-MS only was not possible. The same occurred in the characterization of the floral oil of *Byrsonima sericea*, in which most of the compounds were not identified (Rosa *et al*., 2020).

The compound Geranyl linalool is highlighted as a possible important compound in attracting pollinators in the species of *Byrsonima sericea*, because it is a volatile compound (Rosa *et al*., 2020). In our study, we also find Geranyl linalool in the floral scent samples of *Byrsonima sericea* and *Galphimia brasiliensis* and another volatile compound, Geranyl geraniol, in samples of

Galphimia brasiliensis. However, biotests with oil-collecting bees need to be done to confirm whether these compounds can act as floral attractive.

The composition of the floral scent seems to be related to the type of oil, considering that species rich in acylglycerols produce 1,4 - diacetin in some oil flowers species (Schäffler *et al*., 2015), whereas in Neotropical species the diversity of oil also seems to produce a diversity of volatile compounds. The flower scent composition of the investigated species was species-specific.

The restrict visits of pollinators largely to a single floral type is denominated floral constancy (Waser, 1986; Amaya-Márquez, 2009). Darwin (1876) realized this behavior in bees, that returning to the same flowers type allowed bees to visit food sources more quickly. This specificity can be used by some species of bees that restrict the collection of flower oil to a few species of plants. This could explain, at least in part, the specificity between pollinator and oil flower in the same community (Bezerra *et al*., 2009b), and that some species of oil flowers are visited by few species of bees, while other plant species are visited by many, and different species and genera (Bezerra *et al*., 2009b; Mello *et al*., 2013; Martins, Alves-dos-Santos, 2013; Table 1). It is known that specific blends resembling the composition of selected plant species attract only a few pollinator species (Dodson *et al*., 1969; Williams and Whitten, 1983). This difference in the number of species visitors may be related to the type of compounds found in the floral bouquet of oil flowers, as we found a species-specific floral scent. In summary, the floral traits color and odor are largely variable in species from different Neotropical oil flower families, which may explain the diversity of species of pollinating oil-collecting bee associated with different oil plant species.

2.5 ACKNOWLEDGEMENTS

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2.6 REFERENCES

Alves-dos-Santos I, Machado IC, Gaglianone MC. 2007**.** História natural das abelhas coletoras de óleo. *Oecologia Brasiliensis* **11**:544–557.

Amaya–Márquez M. 2009. Floral constancy in bees: a revision of theories and a comparison with other pollinators. *Revista Colombiana de Entomologia* **35** (2): 206 – 216.

Bezerra ELS. 2008. *Guilda de flores de óleo do Parque Nacional do Catimbau: Fenologia, polinização e sistema reprodutivo*. PhD Thesis, Universidade Federal de Pernambuco, Recife.

Bezerra ELS, Lopes AV, Machado IC. 2009a. Biologia reprodutiva de *Byrsonima gardnerana* A. Juss. (Malpighiaceae) e interações com abelhas *Centris* (Centridini) no Nordeste do Brasil. *Revista Brasileira Botânica* **32** (1): 95-108.

Bezerra ELS, Machado IC, Mello MAR. 2009b. Pollination networks of oil-flowers: a tiny world within the smallest of all the worlds. *Journal of Animal Ecology* 78: 1096-1101.

BRASIL. 2003. Ministério do Meio Ambiente/ Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais renováveis. *Plano de Manejo Reserva Biológica Guaribas*. Brasília.

Brito V, Telles F, Lunau K. 2014. Ecologia Cognitiva da Polinização. In: Rech A, Agostini K, Oliveira PE, Machado IC, eds. *Biologia da Polinização*. Rio de Janeiro: Editora Projeto Cultural.

Brito VLG, Weynans K, Sazima M, Lunau K. 2015. Trees as huge flowers and flowers as oversized floral guides: the role of floral color change and retention of old flowers in *Tibouchina pulchra*. *Frontiers in Plant Science* **6**:362.

Carneiro LT, Aguiar AJC, Martins CF, Machado IC, Santos IA. 2015. *Krameria tomentosa* oil flowers and their pollinators: bees specialized on trichome elaiophores exploit its epithelial oil glands. *Flora* **2015***:* 1-8.

Clarke KR, Gorley RN. 2006. *Primer v6: User Manual/Tutorial,* Plymouth, Primer-E.

Campbell DR, Bischoff M, Lord JM, Robertson AW. 2012. Where have all the blue flowers gone: pollinator responses and selection on flower color in New Zealand *Wahlenbergia albomarginata*. *Journal of Evolutionary Biology* **25**: 352-364.

Chittka L. 1992. The color hexagon – a chromaticity diagram based on photoreceptor excitations as a generalized representation of color opponency. *Journal of Comparative Physiology A: Sensory, Neural and Behavioral Physiology* **170**: 533–543.

Chittka L, Shmida A, Troje N, Menzel R. 1994. Ultraviolet as a component of flower reflections, and the color-perception of Hymenoptera. *Vision Research* **34**:1489–1508 (94) 90151-1.

Chittka L, Kevan PG. 2005. Flower colour as advertisement. In: Dafni A, Kevan PG, Husband BC, eds. *Practical Pollination Biology*. Cambridge Ontario: Enviroquest Ltd, 157–196.

Dados climáticos para as cidades mundiais. CLIMATE-DATA.ORG. 2019. Available: https://pt.climate-data.org/america-do-sul/brasil/pernambuco/camaragibe33802/?fbclid=IwAR2jU8CzGnuX0kyuhIptmOs0aRzTaZKsZh9uwiSo9BDCt1c8-GEEWdX0w. Accessed: Jan 2019.

Darwin C. 1876. On the effects of cross and self fertilization in the vegetable kingdom. John Murray, London, UK. 482 p.

Dodson CH, Dressler RL, Hills HG, Adams RM, Williams NH. 1969. Biologically active compounds in orchid fragrances. *Science* **164**:1234-1249.

Dötterl S, Schäffler I. 2007. Flower scent of floral oil-Producing *Lysimachia punctata* as attractant for the oil-bee *Macropis fulvipes*. *Journal of Chemical Ecology* **33**: 441-445.

Dötterl S, Milchreit K, Schäffler I. 2011. Behavioural plasticity and sex differences in host finding of a specialized bee species. *Journal of Comparative Physiology A* **197**: 1119–1126.

Dyer AG, Chittka L. 2004. Fine colour discrimination requires differential conditioning in bumblebees. *Naturwissenschaften* **91**: 224–227.

Hansen DM, Van der Niet T, Johnson SD. 2012. Floral signposts: testing the significance of visual ‘nectar guides’ for pollinator behaviour and plant fitness. *Proceeding of Royal Society B*. 279634–639

Knauer AC, Schiestl FP. 2014. Bees use honest floral signals as indicators of reward when visiting flowers. *Ecology Letters* **18**: 135–143.

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

Lima PJ, Heckendorff WD. 1985. Climatologia. In: *Governo do Estado da Paraíba. Atlas geográfico do Estado da Paraíba*. João Pessoa: Universidade Federal da Paraíba.

Machado IC. 2004. Oil-collecting bees and related plants: a review of the studies in the last twenty years and case histories of plants occurring in NE Brazil. Pp 255-280. *In:* Freitas BM, Pereira JOP, eds. *Solitary bees, conservation, rearing and management for pollination*. Editora Impressa Universitária, UFCE, Fortaleza. 285.

Malpighiaceae in Flora do Brasil 2020 em construção. Jardim Botânico do Rio de Janeiro. Available: http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB155. Accessed: Ago 2020.

Martins AC, Alves-dos-Santos I. 2013. Floral-oil-producing Plantaginaceae species: Geographical distribution, pollinator rewards and interaction with oil-collecting bees. *Biota Neotropica* **13**: 77-89.

Melo BT, Mota T, Schilindwein C, Antonini Y, Oliveira R. 2018. Floral colour change in *Byrsonima variabilis* (Malpighiaceae) as a visual cue for pollen but not oil foraging by oil-collecting bees. *The Science of Nature* **105**: 46.

Mello MAM, Bezerra EL, Machado IC. 2012. Functional roles of Centridini oil bees and Malpighiaceae oil flowers in biome-wide pollination networks. *Biotropica* **45**: 45–53.

Menzel R. Associative learning in honey bees. 1993. *Apidologie, Springer Verlag* **24** (3):157-168.

Milet-Pinheiro, P, Santos PSC, Prieto-Benítez S, Ayasse M, Dötterl S. 2021. Differential Evolutionary History in Visual and Olfactory Floral Cues of the Bee-Pollinated Genus Campanula (Campanulaceae). *Plants* **10**: 1356.

Ollerton J, Alarcon R, Waser NM. et al. 2009. A global test of the pollination syndrome hypothesis. *Annals of Botany* **103**:1471–1480.

Possobom CCF, Machado SR. 2017. Elaiophores: their taxonomic distribution, morphology and functions. *Acta Botanica Brasilica* 31(3): 503-524.

Proctor M, Yeo P, Lack A. 1996. The natural history of pollination. Timber Press, Portland, Oregon. 479 p.

Rodal MJN, Andrade KVS, Sales MF, Gomes APS. 1998. Fitossociologia do componente lenhoso de um refúgio vegetacional no município de Buíque, Pernambuco. *Revista Brasileira de Biologia* **58**: 517-526.

Renner SS, Schaefer H. 2010. The evolution and loss of oil-offering flowers: new insights from dated phylogenies for plants and bees. *Philosophical Transactions of the Royal Society B* **365**: 423-435.

Rosa FL, Barbosa ABS, Rodrigues THS, Zocolo GJ, Freitas BM. 2020. Chemical characterisation of the floral oil of the Nance (*Byrsonima sericea*): Discovering the constituents used in reproduction by oil-collecting bees. *Sociobiology* **67**(1): 80-88.

Santos JS, Athiê-Souza SM, Almeida NM, Castro CC. 2016. Biologia de flores de óleo em *Cipura paludosa* (Iridaceae). *Rodriguésia* **67**(2): 387-393.

Schäffler I, Balao F, Dötterl S. 2012. Floral and vegetative cues in oil-secreting and non-oil-secreting *Lysimachia* species. *Annals of Botany* **110**: 125-138.

Schäffler I, Steiner KE, Haid M, van Berkel SS, Gerlach G, Johnson SD, Wessjohann L, Dötterl S. 2015. Diacetin, a reliable cue and private communication channel in a specialized pollination system. *Scientific Reports* **5**: 12779.

SUDENE. 1990. *Dados pluviométricos mensais do Nordeste*. Recife, Pernambuco.

Teixeira LAG, Machado IC. 2000. Sistemas de polinização e reprodução de *Byrsonima sericea* DC (Malpighiaceae). *Acta Botanica Brasilica* **14**: 347-357.

Vogel S. 1969. Flowers offering fatty oil instead of nectar. *In:* XI Proc. Int. Bot. Cong. Abstracts. Seattle. 229.

Vogel S. 1974. Ölblumen und ölsammelnde Bienen. *Tropische und Subtropische Pflanzenwelt* **7**: 285-547.

Vogel S, Machado IC. 1991. Pollination of four sympatric species of *Angelonia* (Scrophulariaceae) by oil-collecting bees in NE Brazil. *Plant Systematics and Evolution* **178**: 153-178

Waser NM. 1986. Flowers constancy: Definition, cause and measurement. *The American Naturalist* **127** (5): 593 – 603.

Williams NH. 1983. Floral fragrances as cues in animal behavior, in: Jones, E.C., Little, R.J. (Eds.), *Handbook of experimental pollination biology*. Van Nostrand Reinhold, New York, pp. 50-72.

Zappi D. 2015. Neotropical Malpghiaceae. In: Milliken W, Klitgrd B, Baracat A, eds. *Neotropikey - Interactive key and information resources for flowering plants of the Neotropics*. Available: http://www.kew.org/science/tropamerica/neotropikey/families/Malpighiaceae.htm. Accessed: Aug 202

2.7 SUPPLEMENTARY DATA

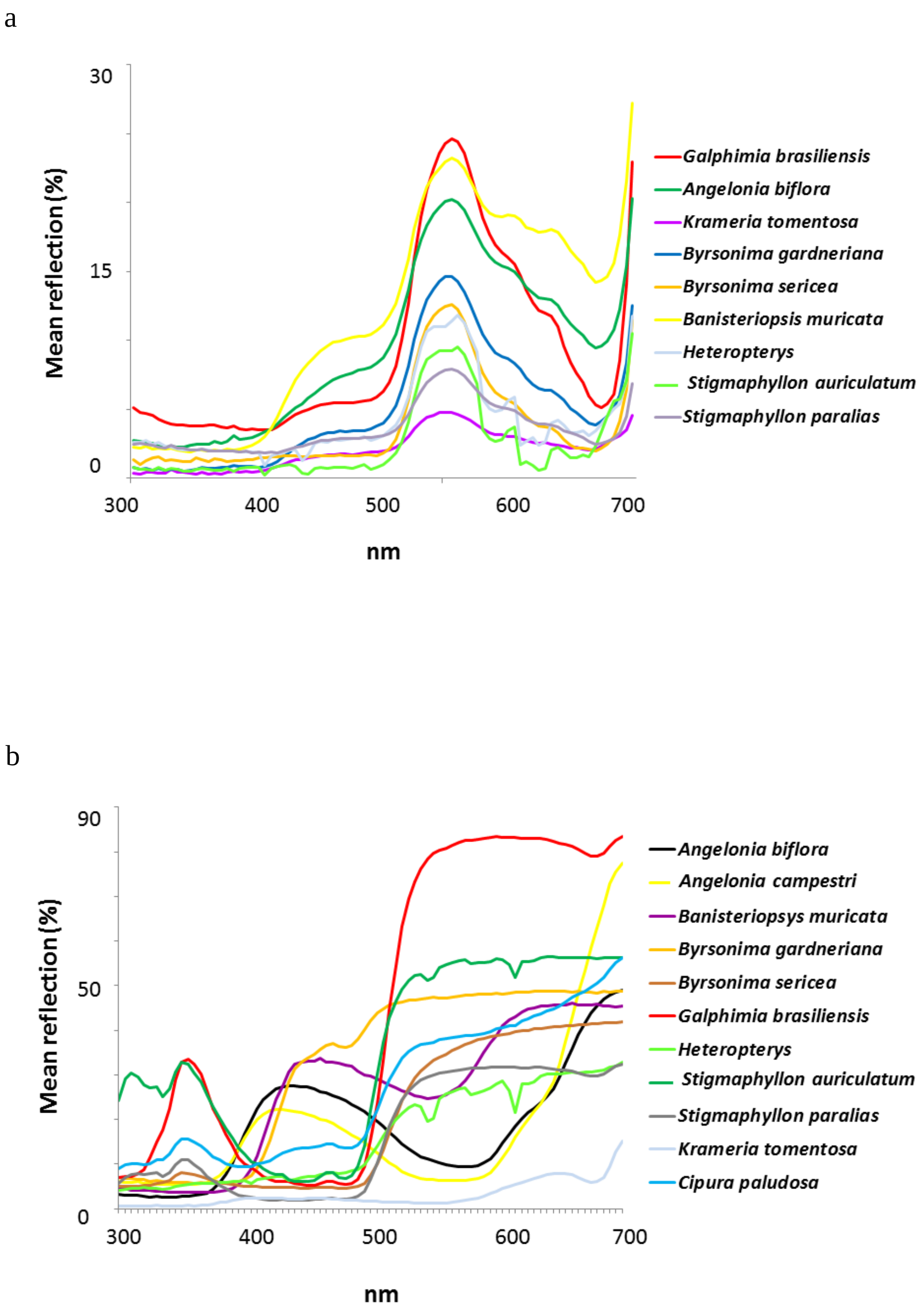

Figure S1 - Mean spectral reflection of (a) leaves and (b) flowers (Flag petal) in eighth oil-floral species (Krameriaceae, Iridaceae, Plantaginaceae and Malpighiaceae). Fonte: Autor, 2021.

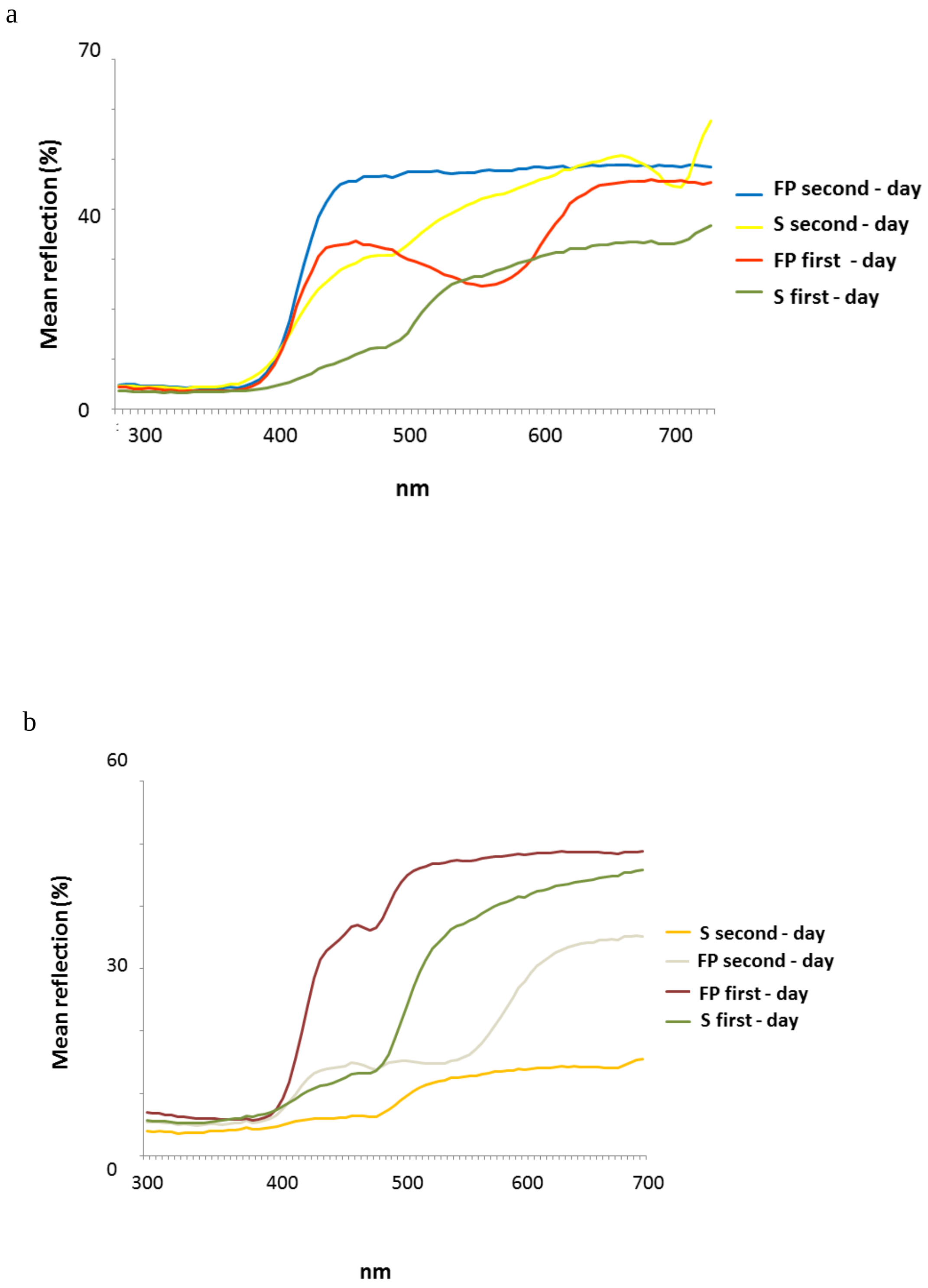

Figure S2 - Mean spectral reflection of flowers of (a) *Banisteriopsis muricata* and (b) *Byrsonima gradneriana* (Malpighiaceae). EA = end anthesis; FP = flag petal; and S = stigmas and stamens. Fonte: Autor, 2021.

TABLE S1. List of identified compounds of flowers and vegetative samples of 14 Neotropical oil plants (Krameriaceae, Plantaginaceae and Malpighiaceae). RI = Retention index. X = compound presence. Kt = *Krameria tomentosa,* Gb = *Galphimia brasiliensis,* Sp = *Stigmaphyllon paralias,* Bys = *Byrsonima sericea,* Byc = *Byrsonima crassifolia,* Byg = *Byrsonima gardneriana,* Sa = *Stigmaphyllon auriculatum, Dp = Diplopterys pubipetala,* Bs = *Banisteriopsis stelaris,* Bm = *Banisteriopsis muricata,* Ab = *Angelonia biflora,* Hb = *Heteropterys byrsonimifolia, Bg = Banisteriopsis gardneriana and* Ca = *Camerea axillares.*

		Kt	*Gb*	*Sp*	*Bys*	*Byc*	*Byg*	*As*	*Dp*	*Bs*	*Bm*	*Ab*	*Hb*	*Bg*	*Ca*
Compound	RI														
Nonanal	1105				X										
Ethylhexanoic acid	1117				X										
cf. Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	1377				X	X									
Unknown (43,56,71,89,173,207)	1378								X						
Caryophyllene <(E)->	1425		X												
(*E*)-β-Farnesene	1457		X												
Germacrene D	1488				X										
1-Pentadecene	1491	X													
Tridecanal	1509	X													

Unknown (34,41,54,67,82,96,208)	1524	X													
1-hexadecene	1593	X													
Unknown (43,57,71,85,111)	1604				X	X									
Tetradecanal <n->	1614														X
Dihydrojasmonate <methyl-, cis->	1658												X		
cf. n-Octyl ether	1664					X									
Unknown (43, 119, 177, 191, 355)	1670	X													
1-Heptadecene	1690	X													
2-Pentadecanone*	1700					X									
Unknown (45,57,69,83,97,210)	1702					X									
1-Pentadecanal	1712	X													
Tetradecanoic acid	1761	X			X										
Unknown (41, 57, 70,83,97,111,228)	1769	X													
Octadecane	1800	X													
cf. hexadecenal	1817				X										X
Neophytadiene Isomer I	1837							X	X	X			X	X	
Neophytadiene	1839													X	X
Hexahydrofarnesyl acetone*	1843			X											
Phytone	1848												X	X	X

Pentadecanoic acid	1859	X													
Galaxolide	1861					X									
Unknown (43,55,68,81,95,96,123,243,278)	1864												X	X	X
Unknown (43,57,68,81,95,109,123,278)	1866									X					
Unknown (43,57,68,82,95,109,123,278)	1885							X	X	X			X	X	X
Unknown (41,55,65,69,109,120,138,262)	1890									X			X		X
Nonadecane	1900					X									
2-Heptadecanone*	1900	X													
cf. n-heptadecanal	1918	X													
sim. Geranyl linalool	1919		X												
Hexadecanoic acid, 1-methylethyl ester	1927	X			X	X					X		X	X	X
Hexadecanolact-16-one	1939	X													
Unknown (41,57,73,85,97,211,229,284)	1941							X							
Unknown (41,55,69,83,96,111,125,236)	1945	X													
sim. Geranyl linalool (squalene)	1953		X												
Pimaradiene	1956		X												
n-hexadecanoic acid	1958	X			X	X			X	X					
sim. A-springene	1968		X												
Unknown (41,57,70,83,97,135)	1981	X													

Sim. Geranyl geraniol	1988		X					
Palmitate <ethyl->	1991							X
9,17-Octadecadienal, (Z)-	1993						X	
Unknown (41, 55,83,98,111, 252)	1993	X						
Unknown (43, 57, 73, 97,111,264)	2004	X						
n-octadecanal	2018	X						
Unknown (43, 71, 97, 139, 237)	2021			X				
Unknown (43,57,71,82,96,110,124,149,250)	2021	X						
Heptadecanoic acid, 1-methylethyl ester	2022	X						
Unknown (43,55,69,83,96,110,236)	2027	X						
Manool oxide<13-epi->	2029					X		
cf. (E,E)-geranyl geraniol	2031		X					
(E,E)-Geranyl linalool	2032			X				
Unknown (41, 93, 107, 135, 257, 272)	2053		X					
Unknown (43,55,69,83,97,111,251)	2081	X						
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	2083						X	
Heneicosane	2100				X			
Unknown (43, 58,85,97,111,282)	2106	X						
Unknown (41, 68, 97, 150, 192, 220)	2121	X						

Unknown (!41,55,67,81,109,123,355)	2133									X	
Unknown (41,55,67,79,97,264,282)	2139									X	
Unknown (40,73,147,221,281,355,429,577)	2140					X		X			
Hexadecanoic acid, butyl ester	2149					X			X	X	
Unknown (43,55,68,81,99,355)	2160									X	
Unknown (43,55,67,81,96,282)	2161										X
Unknown (43,81,96,110,264,284)	2161				X						
Octadecanoic acid *	2164	X		X							
Sim. Geranyl geraniol	2193		X								
Unknown (43,55,73,83,97,111,127,312)	2215	X									
Sim. Geranyl geraniol	2218		X								
Unknown (43,71,97,139,308)	2225			X	X						
Unknown (!43,55,69,83,98,111,143,341)	2286									X	
Unknown (32,73,147,221,281,355,429,535)	2287							X			
Unknown (43,83,98,111,266)	2291			X							
Unknown (43,57,71,85,97,112,141,324)	2297									X	
Tricosane	2300	X			X	X	X		X		
Unknown (43,57,71,83,98,105,123,143,312)	2307	X									
Unknown (41,67,81,95,109,263,336)	2313									X	

Unknown (41,57,67,79,95,334)	2316				X					
Unknown (43, 55, 71, 83, 99, 114, 282)	2348						X			
Unknown (55, 71,99,114,220,276)	2352			X						
Unknown (43,57,69,83,99,114,306)	2353	X								
Unknown (!43,!55,!67,82,!96,109,292)	2354									X
Unknown (55,71,99,114,503)	2356						X			
Unknown (43,55,71,97,115,280,310)	2359			X					X	
Unknown (!43,55,71,81,97,115,334)	2359								X	X
Unknown (43, 55,68,81,96,110)	2360		X							
Hexanedioic acid, bis(2-ethylhexyl) ester	2399	X								
Unknown (41,43,57,82,96,109,306)	2423					X				
Unknown aldehyde (43, 57, 82, 96, 278, 306)	2428	X								
Unknown (73, 147, 221, 281, 355, 429, 595)	2429						X			
Unknown (43,57,82,96,123,504)	2429	X								
Unknown (43, 57, 69, 82, 110, 306, 324)	2431							X		
Unknown (43,55,75,101,129,145,173,188,217,243,283,355,385)	2437								X	
Unknown (43,68,86,95,114,143,278)	2444								X	
Unknown (43, 97,110,123)	2476		X							
Unknown (43,55,69,83, 98, 125, 355)	2488								X	

Unknown (43,55,111,294)	2487			X								
Unknown (43,83,98,111,334,354)	2489					X						
Cf. 1-Docosanol	2490	X			X	X						
Pentacosane*	2500	X			X	X	X	X		X	X	X
Unknown (41, 58, 71, 96, 429)	2514										X	
Unknown (43, 57, 71, 85, 100, 168)	2531				X							
Unknown (!43,55,71,83,97,115,139,308,325)	2559											X
Unknown (43,71,97,115,290,308)	2560					X						
Unknown (43, 55, 71, 99, 114, 331)	2560									X		
Unknown (43,55,71,83,99,125,308)	2563				X							
Unknown (43,55,71,99,503)	2569									X		
Hexacosane <n->	2603								X	X	X	
Docosyl acetate	2610	X										
Unknown (43,57,71,82,110,355)	2627								X			
Unknown aldehyde (43, 57, 82, 96, 334)	2633	X	X									
Unknown (43, 57, 82, 96, 110, 352)	2637										X	
Unknown (43, 70, 95, 306)	2655				X							
Unknown (55,67,81,95,109,137,152)	2667						X					
n-Tetracosanol-1	2667							X				

Unknown (43,55,68,97,125,140,378)	2666											X			
Unknown (57,69,83,97,125,378)	2675											X			
Unknown (41,70,77,105,112,178,204,232,249,360)	2680													X	
Unknown (55,69,83,97,111,125,378)	2681						X								
Unknown (43,57,83,97,111,125,378)	2682							X							
Heptacosane (43, 57, 71, 85)	2700		X			X					X	X	X	X	X
Unknown (32,58,71,97,125,429)	2714			X											
Unknown (43, 59, 71, 85, 366)	2719										X				
Unknown (43,55,81,98,159,237,429)	2728											X			
Unknown (43,71,85,111,125,382)	2730			X											
Unknown (41,57,82,96,109,348)	2733			X											
Unknown (43,57,70,95,149,415)	2750														X
Unknown (43,55,71,97,125,143,405)	2759							X							
Unknown (43,55,68,97,368)	2766											X			
Unknown (43,55,84,99,125,313,396,429)	2781											X			
Octacosane	2800	X	X								X		X	X	X
n-Tetracosyl acetate	2808	X										X			
Unknown (41,69,81,95,137,189)	2813				X										
Unknown (43,57,82,96,111,429)	2830								X						

sim. Squalene	2832		X								
identificavel (43,57,71,82,362)	2837		X								
Unknown (43,69,81,99,125,199,291,429)	2841								X		
Unknown (43,57,81,142,171,405)	2859								X		
Unknown (43,57,83,97,111,139,406)	2872				X	X					
Nonanoic acid, octadecyl ester	2878								X		
Unknown (57,69,97,111,125,429)	2882				X						
Unknown (43,55,83,97,111,139,406)	2890				X						
Nonacosane	2900							X	X	X	X
Unknown (31,59,97,111,394)	2918	X									
UnKnown (43,55,96,159,265,503)	2928								X		
Unknown (43, 57, 97, 123, 420)	2930			X							
Unknown (43,74,87,143,410)	2940					X					
Unknown (43, 68, 97, 141,334)	2979			X							
Unknown (43,57,84,111,129,341,424,503)	2981								X		
Unknown (43,55,95,135)	2985			X							
Unknown (43, 57, 71, 99, 113, 422)	2990						X	X			
Unknown (43,99, 143, 222,336)	2991			X							
Unknown (43,57,81,96,125,143,199,424)	2994								X		

Triacontane	3000	X	X											X	X
Unknown (43, 57, 71, 99, 113, 422)	3001										X				
Unknown (57,71,85,99,127,142,422)	3003									X					
Unknown (68,81,97,140,169,429)	3007					X									
Unknown (43,57,83,97,111,424)	3009											X			
Unknown (43, 57, 83, 97, 111, 207, 364)	3011		X												
Unknown (43, 142, 153, 171,222,336)	3017				X	X						X			
Unknown (43,57,82,96,110,390)	3037	X													
Unknown (43,57,82,96,111,390)	3040		X			X									
Unknown (41,69,189,203,218,429)	3046					X									
Unknown (41,189,203,218,408)	3047													X	
Unknown (41,55,69,95,119,203,218,408)	3053									X					
gamma.-Tocopherol	3057	X													
Unknown (41,55,203,218,408)	3059													X	
Unknown (41,69,189,218,257,408)	3059					X									
Unknown (32,73,147,221,295,503)	3066												X		
Unknown (43,67,82,96,124,138,432)	3069								X						
Unknown (43,57,83,97,125,139,434)	3071							X		X					
Unknown (43,55,159,265,499)	3074											X			

Unknown (43, 101, 117,145,217,410)	3078					X									
Unknown (41, 57, 83, 97, 111, 432)	3080										X				
Unknown (41,43,71,95,108,141,157,309,422)	3080								X						
Unknown (43,67,82,96,124,432)	3083							X							
Unknown (43,57,83,97,125,434)	3085				X										
Unknown (43, 67, 79, 108, 430)	3090										X				
Unknown (41,189,218,408)	3092					X									
Unknown (43,57,79,108,155,295,434)	3093							X							
Hentriacontane	3100		X			X	X				X	X		X	X
Unknown (57, 73, 85, 109, 218, 393)	3115	X													
Unknown (55,95,119,189,203,218,408)	3116					X									
Unknown (43,79,105,121,147,218,339,408,429)	3122					X									
Unknown alcohool (59, 71, 97, 111, 395, 422)	3122	X													
Unknown (43,55,81,98,159,293,408)	3127											X			
Unknown (43, 97, 123, 448)	3129				X										
Unknown (43,74,87,111,143,438)	3139								X						
Unknown (55,69,93,122,189,203,218,408)	3164					X									
Unknown (43,57,83,112,129,341,452)	3174											X			
Unknown (57,97,127,145,452)	3178				X		X								

Unknown (43,57,84,97,129,145,369,452)	3183											X		
Unknown (43, 71, 97, 117)	3187		X											
Dotriacontane	3200	X	X											X
cf. n-Octacosyl acetate	3213	X												
Unknown (43,57,83,97,125,254,409)	3223	X												
Unknown (43, 142, 153,171,250,364)	3228				X	X								
Unknown (57,82,143)	3234				X									
Unknown (43, 57, 85, 491)	3245										X			
Unknown (43, 57, 82, 96, 111, 151, 418)	3246		X											
Unknown (55, 82, 96, 124)	3262				X									
Unknown (41,57,69,95,109,135,175,381,430)	3264			X										
Unknown (43,57,127,145,466)	3269				X									
Unknown (43,159,293,469)	3270											X		
Unknown (43,67,82,96,124,460)	3272								X					
Unknown (43,67,82,96,124,460)	3278				X									
Unknown (43,57,84,129,383,466)	3281											X		
Unknown (41,57,82,96,124,138,460)	3284							X						
Unknown (43,67,79,108,458)	3289			X										
Unknown (41,43,79,108,458)	3295										X			

Unknown (43,57,85,99,127,405)	3296	X								
Tritiacontane	3300			X				X		X
Unknown (32,69,83,97,111,138,153,197,422)	3307	X	X							
Unknown (!57,69,97,111,125,420)	3312				X					
Unknown (41, 69, 95, 109, 205, 315)	3318			X						
Unknown (41,67,81,96,123,462)	3321					X				
Unknown (43,59,83,97,125,155,450)	3324	X								
Unknown (43,55,81,117,159,291,466)	3325								X	
Unknown (69,93,109!,205,313,424)	3325			X						
Unknown (81, 109, 133, 205, 218, 422)	3329		X							
Unknown (203, 218,409)	3334			X						
Unknown (57,93,109,205,414,426)	3335				X					
Unknown (95, 109, 189, 256, 409, 424)	3335		X							
Unknown (41, 55, 69, 95, 119, 189, 203, 218, 424)	3337						X			
Unknown (41,69,93,109,207,426)	3338			X						
Unknown (41,95,119,135,189,203,218,409,424)	3339									X
Unknown (43,57,74,97,129,143,466)	3339					X				
Unknown (41,69,81,109,121,135,189,207,426)	3340									X
Unknown (95, 119, 189, 203, 218, 426)	3341		X							

Unknown (41,55,69,95,109,189,203,218,424)	3346						X			X
Unknown (55,69,95,107,135,175,189,204,287,302,426)	3348	X								
Unknown (82,96,127,145,334)	3351		X							
Unknown (41,69,95,109,135,189,207,426)	3351									X
Unknown (32,55, 67, 121, 149, 177, 204, 409, 424)	3354						X			
Unknown (43,55,81,136,159,290,466)	3355							X		
Unknown (43,67,81,109,145,476)	3362				X					
Unknown (189, 203, 218, 426)	3366		X						X	X
Unknown (69,93,109,207,426)	3369		X							
Unknown (67,79,93,108,426)	3373				X					
Unknown (43,57,83,112,129,369,480)	3376							X		
Unknown (80,93,109,136,405,426)	3376			X						
Unknown (41,55,189,203,218,424)	3376									X
Unknown (57, 97, 127, 145,481)	3380		X							
Unknown (57,97,111,145,381,480)	3381				X					
Unknown (43,57,!84,111,129,397,480)	3383							X		
Unknown (55, 83, 97, 119, 257, 423. 438)	3384						X			
Unknown (96,124, 218)	3385		X							
Unknown (57,83,97,127,145,480)	3386					X				

Unknown (189, 203, 218, 424)	3388		X										X	X
Unknown (55,95,123,299,381,409)	3393			X										
Unknown (41,67,95,109,205.424,503)	3394									X	X			
Unknown (189, 203, 218, 424)	3410		X										X	X
Unknown (41,55,69,95,286,426)	3411								X					
Unknown (95,121,245,340,423)	3445			X										
Unknown (43, 69, 81, 95, 121, 147, 161, 201, 438)	3450									X	X			
Unknown (57, 83, 105, 123, 218, 458)	3451		X											
Unknown (43,55,81,95,121,147,175,407,425)	3454													X
Unknown (95,121,175,407,425)	3458			X										
Unknown (203,218,412,468)	3460				X	X								
Unknown (43, 55, 69, 95, 124, 175, 203, 218, 412, 468)	3466										X			
Unknown (43, 55, 95, 121, 161, 177, 189, 468)	3474										X			
Unknown (32,41,69,95,109,229,247,426)	3479	X	X											
Unknown (95,203,218,53,468)	3503					X								
Unknown (81,107,189,218)	3504				X									
Unknown (43, 55, 69, 95, 121, 189, 218, 468)	3509										X			
Unknown (43,57,81,109,126,150,490)	3509	X												
Unknown (41, 55, 69, 95, 123, 137, 426)	3538										X			

Unknown (43, 69, 109, 123, 424)	3542				X	X						
Unknown (55,67,81,109,153,502)	3550						X					
Unknown (43, 69, 105, 123, 220, 405)	3553		X									
Unknown (69,109,207,313,426)	3566					X	X					
Unknown (43,57,97,111,140,369,508)	3571											X
Unknown (43, 109, 189, 426)	3571				X							
Unknown (57,71,97,112,397,508)	3577											X
Unknown (43,57,97,111,155,173, 509)	3581							X				
Unknown (43,57,84,145,425,508)	3584											X
Unknown (43,57,97,111,145,508)	3587							X				
Unknown (133,189,203,232,346)	3635					X						
Unknown (57, 83, 105, 123, 218, 486)	3653		X									
Unknown (43,57,95,126,150,178,207,518)	3713!	X										

TABLE S2. Posteriori pairwise comparisons and analysis of similarity (ANOSIM) of oil flowers species (Krameriaceae and Malpighiaceae) R= 0.95, p = 0.001.

Groups	R	P
K. tomentosa, G. brasiliensis	0.85	0.001
K. tomentosa, S. paralias	0.83	0.001
K. tomentosa, B. sericea	0.96	0.001
K. tomentosa, B. crassifolia	0.98	0.001
K. tomentosa, B. gardneriana	0.79	0.002
G. brasiliensis, S. paralias	0.69	0.002
G. brasiliensis, B. sericea	0.92	0.001
G. brasiliensis, B. crassifolia	0.92	0.002
G. brasiliensis, B. gardneriana	0.53	0.001
S. paralias, B. sericea	0.90	0.001
S. paralias, B. crassifólia	1	0.001
S. paralias, B. gardneriana	0.64	0.001
B. sericea, B. crassifólia	0.99	0.002
B. sericea, B. gardneriana	0.37	0.002
B. crassifolia, B. gardneriana	0.67	0.002

Fonte: Autor, 2021.

TABLE S3. *Posteriori pairwise comparisons of oil plants. Analysis of similarity (ANOSIM) of oil flowers species (Krameriaceae and Malpighiaceae) R= 0.54, p = 0.001.*

Groups	R Statistic	P
Krameria and *Galphimia*	1	0.002
Krameria and *Stigmaphyllon*	1	0.001
Krameria and *Byrsonima*	0.522	0.001
Galphimia and *Stigmaphyllon*	1	0.001
Galphimia and *Byrsonima*	0.489	0.001

Fonte: Autor, 2021.

3 ARTIGO 2 - Floral signals of the dimorphic oil-producing *Byrsonima sericea* (Malpighiaceae): is the eglandular morph deceptive?

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Running title: Floral cues and deceptive oil bee pollination

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Background and Aims: The loss of floral oil production within Malpighiaceae is commonly associated with their historical expansion from the Neotropics to the Paleotropics, where their oil-collecting pollinators are absent. However, the reduction or loss of oil glands within several neotropical species is intriguing and thought to be related to the energetic costs of oil production and deceptive pollination mechanisms. Here, we evaluated whether the loss of oil production in eglandular individuals of the dimorphic *Byrsonima sericea* generates deceptive mechanisms in oil bee pollination.

Methods: We monitored the flower visitors and quantified the floral cues and reproductive success associated with glandular and eglandular morphotypes of *B. sericea*. The reflectance of floral parts of both morphs was measured and modeled with the bee color hexagon. Floral volatiles were collected by two different methods (dynamic headspace and solvent extraction), and compared between morphs.

Key results: Both morphs were similar in flower-visiting assemblage, reproductive success (fruit set) and floral reflectance. The analyses of the headspace samples indicated no between-morph differences in floral volatiles, whereas flower extracts showed a clear difference in chemical composition.

Conclusions: The absence of oil production in some individuals of *B. sericea* was not associated with changes in floral cues used in pollinator attraction at long distances. This may cause a deceptive mechanism that triggers the oil-collecting behavior of bees even in the absence of oil glands. However, a higher female reproductive success was not observed in the eglandular morphotype, suggesting at least that energetic costs associated with oil production are not allocated for fruit set.

Keywords: **Floral scents, Floral visual cues, Oil-collecting bees, Oil flowers, Pollinator deceit.**

3.1 INTRODUCTION

Within some angiosperm families, such as Krameriaceae, Malpighiaceae and Plantaginaceae, tens to hundreds of species have evolved a remarkable pollination strategy, in which pollinators seek for floral oils (Vogel, 1974; Vogel and Machado, 1991). This interaction was first described for several of those species approximately 50 years ago by Stefan Vogel (1969), who observed secretions of hydrophobic substances (fatty acids) through specific floral glands, which he named as elaiophores. He pointed out that those species (hereafter oil plants) are pollinated by oil-collecting bees (hereafter oil bees) (Vogel, 1969), which are most representative in the neotropical tribe Centridini (Apidae). Oil as a floral reward emerged independently at least 28 times in Angiosperms (Renner and Schaefer, 2010). Malpighiaceae originated in the Neotropics and represents the oldest and the most diverse plant clade presenting elaiophores (Vogel, 1974; Renner and Schaefer, 2010). Interestingly, the loss of these glands occurred more recently over the evolutionary history of the family (Vogel 1974, 1990).

Most representatives of Malpighiaceae have a conserved floral morphology that comprises zygomorphic flowers, which bear sepals with abaxial sides covered by a pair of elaiophores (Vogel, 1990; Anderson, 1990; Davis *et al*., 2014). Changes in the expression of the CYC2 gene in more recent lineages of Malpighiaceae have led to the emergence of a differentiated floral morphology, which is represented by the loss of elaiophores and adaptations to pollen rewarding. This derived condition of the family appears in representatives from the Paleotropics, where Centridini oil bees are absent (Davis, 2002; Davis and Anderson, 2010; Zhang *et al*., 2010, 2012, 2013; Davis *et al*., 2014).

The conserved floral morphology present in the neotropical Malpighiaceae is highly associated with the stereotyped oil-collecting behavior of Centridini bees (Vogel, 1990). During the visits, these bees grasp the flag petal with their mandibles and perform several scraping movements with their fore- and mid-legs over the elaiophores. This oil-collecting behavior have been pointed out as essential for plant reproductive success (Sigrist and Sazima, 2004; Aliscioni *et al.*, 2018; Carneiro *et al.*, 2019). In Malpighiaceae, both oil and pollen can be rewards for oil bees, and several neotropical species have completely lost the elaiophores, becoming pollen flowers (Sazima and Sazima, 1989; Vogel, 1990). Other variations occurring in neotropical representatives involve poly- and dimorphisms associated with reductions in number or complete absence of elaiophores within populations (Costa *et al.*, 2006).

In dimorphic oil plants, such as *Banisteriopsis muricata, Heteropterys aceroide* (Sazima and Sazima, 1989) and *Byrsonima sericea* DC. (Teixeira and Machado, 2000), a fraction of individuals in the populations has no elaiophores (eglandular morphotype). Sazima and Sazima (1989) suggested that individuals with eglandular flowers "mimic" individuals with glandular flowers and deceit floral oil-seeking bees, while saving energetic costs from oil production. However, the ecological outcomes involved in the reduction or absence of oil production in individuals of oil-producing species have been poorly understood. Individuals without or with a reduced number of glands are known to allocate more resource to pollen production (Teixeira and Machado, 2000). Nevertheless, even if oil bees can collect both oil and pollen on the flowers (Teixeira and Machado, 2000; Schäffler *et al.*, 2012; Melo *et al.*, 2018), individual plants that offer only pollen as reward for their pollinating bees would be also expected to face reproductive uncertainties due to reductions in pollen delivery (Cruden, 2000). Therefore, the maintenance of floral cues (*e.g.*, visual and olfactory) associated with oil offering in eglandular individuals should be advantageous since they should trigger deceptive mechanisms related to the oil-collecting behavior (Sazima and Sazima, 1989). Nevertheless, the emission of similar olfactory cues by eglandular individuals should be harmed as the synthesis of specific floral scents that attract oil-seeking bees may be connected to oil production (Schäffler *et al.,* 2015).

The floral cues involved in plant–pollinator communication in floral oil systems have been investigated only for a few species (Dötterl *et al*., 2011; Melo *et al*., 2018; Schäffler *et al*., 2012, 2015; Albuquerque, 2021). Studies have demonstrated that both visual and olfactory signals are important in locating oil flowers, and these signals are likely to be subject of pollinator-mediated selection (Dötterl *et al*., 2011; Schäffler *et al*., 2012). For instance, in *Byrsonima variabilis* A. Juss. (Malpighiaceae), the visual cues may indicate the availability of pollen (Melo *et al*., 2018). This signal can be interpreted as a foraging filter in some plant species, leading bees to approach the flowers. However, the recognition of resources can also be signalized by the floral scent (Milet-Pinheiro *et al*., 2015). When investigating the floral signals involved in the recognition of *Lysimachia* oil plants by *Macropis* Panzer oil bees, Schäffler *et al*. (2015) reported an interesting case of private communication channel between these mutualists. 1,4-Diacetin, which is a quite rare compound found within angiosperms, is a common constituent of floral scents in oil-producing species. However, it is still unknown whether certain compounds are strictly associated with floral oil production.

In this study, we tested the hypothesis that the loss of oil secretion in pollen-rewarding (eglandular) individuals of *B. sericea* is adaptive through pollinator deception, which should be associated with the emission of floral cues that are similar to those found in individuals with glandular flowers. For this, we compared the reproductive success and flower visitation pattern of both morphs as well as their floral reflectance properties and floral scent composition. Visual cues are expected to be similar between morphs, since there are no apparent evidences for human perception of within-population variation in the flower reflectance of this species. However, some floral scent compounds are expected to be found only in the glandular morphs in association with floral oil biosynthetic routes. We also addressed the following three main questions: 1) Does the reproductive success of *B. sericea* vary according to morphotype? 2) Are the morphotypes associated with the same pollinators, providing similar flower visitation patterns? 3) Are the floral cues of the two morphs similar?

3.2 MATERIALS AND METHODS

3.2.1 Study sites and plant species

The study was conducted in fragments of Atlantic Rain Forest in Aldeia, Pernambuco (7º56’14” S, 35º01’57” W, from now on named Aldeia), and in the *campus* I of the Federal University of Paraíba, in João Pessoa, Paraíba (7º08’16’S, 34º51’19’’W, from now on *Campus* I), both in Northeastern Brazil within the Atlantic Rainforest domain. The climate is warm and humid, with annual average temperature close to 25º C and annual total precipitation ranging between 1,500 and 1,700 mm (climate-date-org, 2019; Lima and Heckendorff, 1985).

Byrsonima sericea (Fig.1) is a woody species native to South America and widely distributed throughout Brazilian phytogeographic domains (Flora do Brasil, 2019). The individuals reach 3-20 m in height and bloom from October to February. Both glandular and eglandular morphs can be found within the populations of *B. sericea* (Teixeira and Machado, 2000). In Atlantic Rain Forest, the morphs occur in the 1:1.5 ratio (Rosa *et al*., 2007). This species is self-incompatible and thus cross-pollen transfer by pollinating bees are required to a successful reproduction. In both morphs, the inflorescences are terminal, containing approximately 45-60 flowers. The flowers are hermaphrodite and zygomorphic. The corolla is yellow according to human perception. The gynoecium is 3-carpelar and divides into three locules, each one bearing a single ovule. The three long styles are free and acute at the apex and exceed the upper limit of the anthers (Teixeira and Machado, 2000).

3.2.2. Flower visitation surveys

Flowers of both glandular and eglandular morphs were monitored in order to quantify and identify flower visitors, and to describe their foraging behavior (oil and/or pollen gathering). The field observations were performed in two fragments of Atlantic Forest, (Aldeia and *Campus* I), between 06h00 and 16h00, with 12 h of observation in the morning (06h00-12h00) and 8h in the afternoon (12h00-16h00), for both morphs. In the first applied protocol, the observations were performed in December 2018 and January 2019 for eglandular flowers and in January and February 2019 for glandular flowers. During the observation period on different days two individuals were observed for the glandular morph and three individuals for the eglandular morph; in each individual 12 inflorescences were observed. The sampling protocol was adjusted in the subsequent flowering season for better comparisons of the composition of visitors between morphs. This protocol was performed in three days in November 2019, four hour for each day. The observations occurred between 06h00 and 12h00 for each morph, with two-hour intervals interspersed on the same day for the different morphs. The individuals of different morphs were close, approximately 200 meters, to each other in the study area. In total, in the protocols together, flower visitors were recorded for 52-h observations (26 hours for each morph).

3.2.3. Comparisons of the reproductive success between morphs

In order to test whether the reproductive success of *B. sericea* differs between morphs, the fruit set of glandular and eglandular individuals were quantified in both study sites. One to three inflorescences containing a mean of 40 (±13) flowers were tagged in eight glandular (17 inflorescences and 604 flowers) and seven eglandular (19 inflorescences and 832 flowers) individuals. The occurrence of fruit development was observed for 1436 flowers in total after 120 days approximately. Differences in fruit set between morphs were evaluated using R v3.6.2 (R Core Team, 2019) through generalized linear mixed models (GLMM, *lme4* package) for binomial distribution (occurrence and non-occurrence of fruit formation) (Bates *et al.*, 2015). A nested hierarchical structure with tagging date, study site and individual plant was included as random effects in the models. A model containing morphotype as fixed effect was contrasted against the null model with ANOVA to test for variation in fruit set between morphotypes. Predicted probabilities and confidence intervals for fruit set were computed with the *lsmeans* package (Lenth, 2016).

3.2.4. Comparisons of flower reflectance between morphs

The color reflectance of flowers and young leaves (leaves close to flowers) belonging to each *B. sericea* morph was quantified with a spectrophotometer (Jazel200, Ocean Optics, USA) (Campbell *et al.*, 2012). Using five individuals per morph and a single flower per individual, the reflectance of the following floral whorls were measured: 1) standard petal, 2) lateral petals, 3) basal petals and 4) stamens and stigmas. These floral parts were selected in order to represent the entire front floral display. Leaves of each individual were measured to represent the background of the flowers. To test for differences between morphs, reflectance curves of each floral part were compared with multivariate analyzes (ANOSIM). Nonmetric multidimensional scaling (NMDS) was used to display graphically the reflectance curves. ANOSIM and NMDS were run in the software PRIMER 7. In order to understand how color of different morphs (and different floral parts) is processed through bee vision, the reflectance curves were plotted in the bee color hexagon, which is currently the most accepted model (Chittka, 1992). The color loci plotted in the hexagon are considered distinguishable by bees when distances between the loci are greater than 0.1 hexagon units (Chittka *et al.*, 1994).

3.2.5 Collection of floral scent compounds

Flower extracts - For the chemical characterization of the floral scents of both morphs of *B. sericea*, floral extracts were collected using hexane (*n* = 10 samples from different individuals; five samples per morph). This is the same method previously used to characterize the odor emitted by other floral oil-secreting plants (Schäffler *et al.*, 2015). Five flowers per individual were excised and inserted into the same 1.5 ml (Analitica) vial filled with 1 ml of hexane representing a single sample. In the morning, flowers were immersed in hexane during two minutes and then removed from the vials. The samples were filtered with glass wool and concentrated with nitrogen gas flow until the volume reached 0.5 ml. The same protocol was performed with young leaves (close to the flowers). Three leaves were used to make one sample. For each morph, we collected one sample of leaves.

Dynamic headspace – Inflorescences (ten per sample) from five glandular and five eglandular individuals were individually enclosed by a polyester oven bag (Toppits®, Germany) during 1 h for scent retention. After this, the scent inside the bag, enriched with the floral volatile compounds was drawn for 10 min through an adsorbent filter, which was connected to a membrane pump using silicone tubing (G12/01 EB, Rietschle Thomas, Puchheim, Germany). The pump worked at a constant flow rate of 200 $ml.min^{-1}$. The adsorbent filter consisted of glass tubes (length: 30mm; inner diameter: 2.5 mm) filled with 3 mg of a 1:1 mixture of Tenax-TA (mesh 80/100; Supelco, Bellefonte, Pennsylvania, USA) AND Carbopack X (mesh 20/40, Sepelco, Bellefonte, Pennsylvania, USA), which was held in the tubes using glass wool. To determine contaminants in the surrounding environment, samples of fresh air (empty bags, n = 3) and leaves (n = 3) were collected during 30 min and trapped over 10 min as controls. Compounds found in vegetative and air samples were excluded from the list of floral compounds.

3.2.6. Identification of floral scent compounds

Both floral extracts and floral headspace samples (samples for thermodesorption) were analyzed with gas chromatography coupled to mass spectrometry (GC-MS; Agilent 7890A™ gas chromatograph, Agilent 5975C Series MSD™ mass spectrometer, Palo Alto, USA), equipped with a non-polar HP-5ms™ column (Agilent J&W; 30 m x 0.25 mm d.i., 0.25 µm film thickness) and a thermal separation probe (TSP, Agilent, Palo Alto, USA). Aliquots of the flower extract (1µL) were placed in a microvial, which was loaded into the probe and inserted into a modified GC injector. For flower headspace samples, the chromatoprobe was also loaded into the probe and inserted into a modified GC injector. The GC injector worked under 300 ºC. The temperature of the GC oven was set at 40 ºC during 1 min, with subsequent increasing at a rate of 6ºC min^{-1} until 290ºC. The compounds were identified through GCMS Solution Version 2.72 (1999-2012). The identifications were conducted by comparing the mass spectra and retention indices obtained from the samples with reference samples available in commercial libraries (Adams, Essential Oils 23P, FFNSC 2, STANDARDSSA, W9N11). The relative amount of compounds was calculated as a ratio between the total ion current (TIC) of each compound and the sum of TICs of all compounds in the sample. The same compounds found in the surrounding environment, including those emitted by the leaves, were excluded from the list of floral scent compounds.

3.2.7. Comparison of floral scent compounds between morphs

Between-morph differences in the chemical composition of floral scents were tested through an ANOVA of similarity (ANOSIM), using a *Bray-Curtis* dissimilarity matrix, followed by a posterior pairwise comparison. In order to depict the (dis)similarities in floral scent bouquet between morphs, a multidimensional Scaling (MDS) analyses was performed.

3.3. RESULTS

3.3.1. Flower visitors

We recorded seven bee species and one wasp visiting flowers of *B. sericea* (Table 1; Fig. 1). The bees collected pollen and/or oil from both floral morphs, while the unidentified wasp was observed visiting only eglandular flowers. The bees *Centris* sp.1, *Centris cf. aenea* and *Epicharis* sp.1, visited glandular morphs and collected oil, contacting the reproductive structures (*i.e.*, anthers and stigmas); *Augochlorini* sp., collected pollen, and also contacted the reproductive structures; *Tapinotaspidini* sp. collected oil, but did not contact the reproductive structure, and *Trigona sp.* collected pollen, but acted as a robber, damaging buds in pre-anthesis stage, without pollinating the flowers. *Augochlorini* sp., *Epicharis* sp.1, and *Epicharis* sp. 2, collected pollen on the eglandular flowers and contacted their reproductive structures, whilst *Trigona* sp. and an unknown bee also collected pollen, but acting as a robber. Prior to pollen collection on the eglandular flowers, *Epicharis* species triggered the same grasping and scraping movements usually performed when collecting floral oils on glandular flowers.

TABLE 1. *Flowers visitors of glandular and eglandular flowers of* Byrsonima sericea *in fragments of rain forest (Atlantic Forest)*

Flower visitors	Glandular flowers	Eglandular flowers	Resource collecting	Result of visit
Centris sp. 1	15.5% (45)		Oil / Pollen	Pollination
Centris cf. aenea	17.7% (45)		Oil / Pollen	Pollination
Epicharis sp.1	8.8% (45)	9.3% (32)	Oil/Pollen	Pollinator
Epicharis sp.2		62.5% (32)	Oil / Pollen	Pollinator
Unknown bee		9.3% (32)	Pollen	Robbering
Tapinotaspidini sp.	6.6% (45)		Oil	Robbering
Trigona sp.	44.4% (45)	3.1% (32)	Pollen	Robbering
Augochlorini sp.	6.6% (45)	12.5% (32)	Pollen	Pollinator
Apoidea (wasp) sp1		3.1% (32)		

Fonte: Autor, 2021.

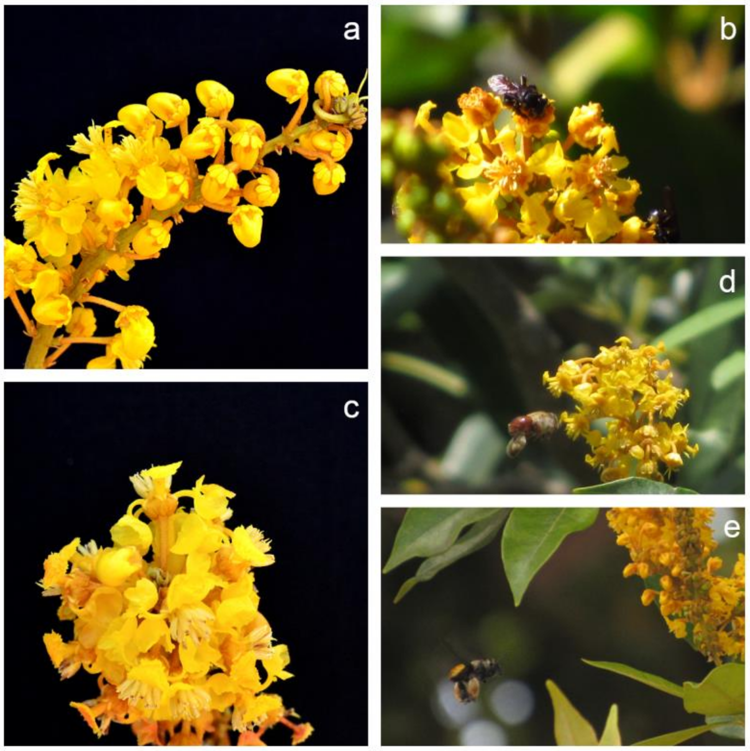


Figure 1 - General overview of flowers and floral visitors of *Byrsonima sericea* (Malpighiaceae), glandular flowers and floral buds (a); *Trigona* sp. in glandular buds (b); eglandular flowers (c); *Centris* sp. approaching glandular flowers (d) and female of *Epicharis bicolor* approaching eglandular flowers (e), in fragments of Atlantic Forest. Fonte: Autor,2021.

3.3.2. Reproductive success

Differences in the probability of setting fruits were not detected between both morphotypes (glandular and eglandular) of *B. sericea* ($\chi^2 = 0.26$; $df = 1$; $p = 0.606$, Fig. 2). The predicted fruit set was equivalent to 17.5% and 13.6% for inflorescences belonging to the glandular and eglandular morphs, respectively, considering the structure of our sampling design (Fig. 2).

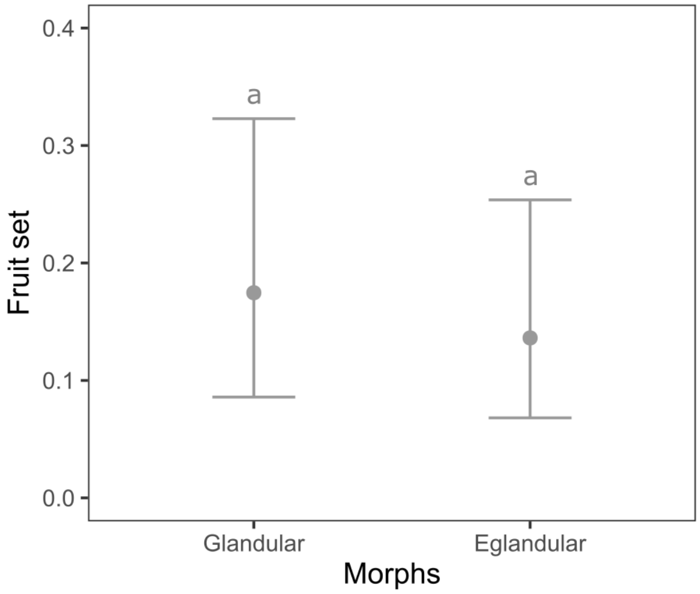


Figure 2 - **P**redicted probabilities (and confidence intervals) of the reproductive success (measured as fruit set) quantified in both glandular and eglandular morphs of *Byrsonima sericea* (Malpighiaceae) in fragments of Atlantic Rain Forests from northeastern Brazil. Same letters represent similarities in fruit set between both morphs. Fonte: Autor, 2021.

3.3.3. Flower reflectance

Glandular and non-glandular flower petals have reflectance at UV wavelength (300 - 400 nm), with the highest peak at length 500 - 700 nm. Stamens and stigmas of both morphs reflect from 400 nm, but the peak is found between 500 and 700 nm (Fig. S1). The leaves have reflectance peaks between 500 and 600 nm (Fig. S2). The distances between loci lower than 0.1 in the color hexagon indicated that the pollinators cannot distinguish floral morphs (Fig. 3).

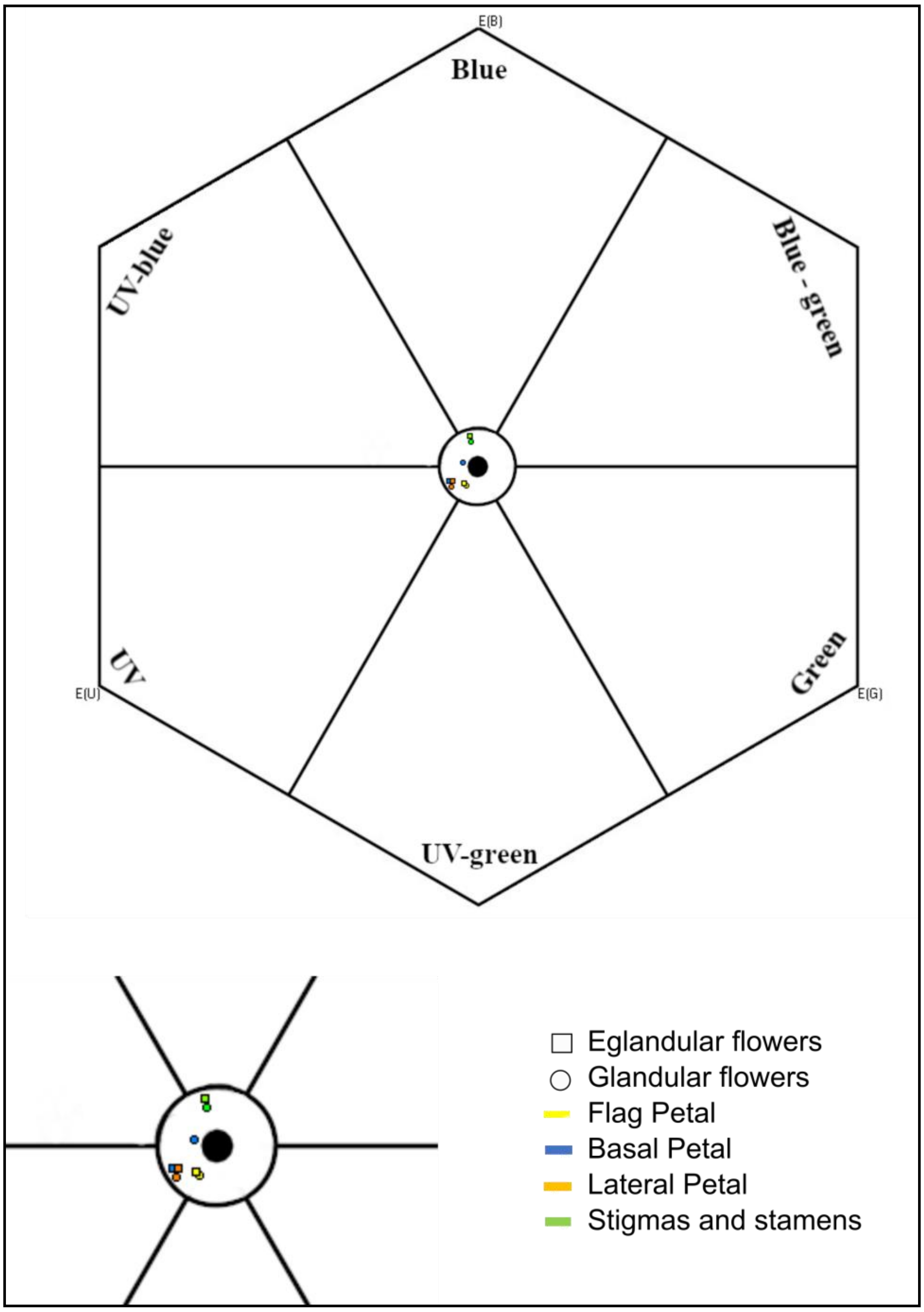


Figure 3 - Colour of floral parts of *Byrsonima sericea* (Malpighiaceae) with glandular and eglandular flowers in the model hexagon vision of the bees. UE, EB, EG: excitation of the UV receptor, blue and green, respectively. Fonte: Autor, 2021.

Multivariate analyses revealed no difference in color reflectance properties between morphotypes, considering (1) flag petals (ANOSIM: *r* = -0.16, *p* = 1), (2) lateral petals (*r* = -0.15, *p* = 0.960), (3) basal petal (*r* = -0.05, *p* = 0.651) and (4) stamens and stigmas (*r* = -0.04, *p* = 0.532). The same was also found for leaves (*r* = -0.06, *p* = 0.659). The absence of differences in the reflectance of floral parts between morphs is evident when modelling the reflectance curves in the bee hexagon.

3.3.4. Chemical characterization of flower scent

Flower extracts - The glandular flowers had saturated and unsaturated hydrocarbons also found in leaves and eglandular flowers, but the former had several additional lipidic compounds (Table 2). The ANOSIM analyses revealed that the chemical composition of floral extracts differed between glandular and eglandular flowers, and between flowers and leaves in *B. sericea* (ANOSIM, *r* = 0.61; *p* = 0.001). The *a posteriori* pairwise comparisons indicated that eglandular and glandular flowers, as well as the leaves significantly differ among each other (eglandular vs. glandular flowers, *r* = 0.55; *p* = 0.016; eglandular flowers vs. leaves, *r* = 0.81; *p* = 0.048 and glandular flowers vs. leaves, *r* = 0.68; *p* = 0.048) (Table S1) (Fig. 4).

TABLE 2. Scents compounds of glandular and eglandular flowers and leaves of Byrsonima sericea *(Malpighiaceae). RI = Retention index. X = presence of the compound*.

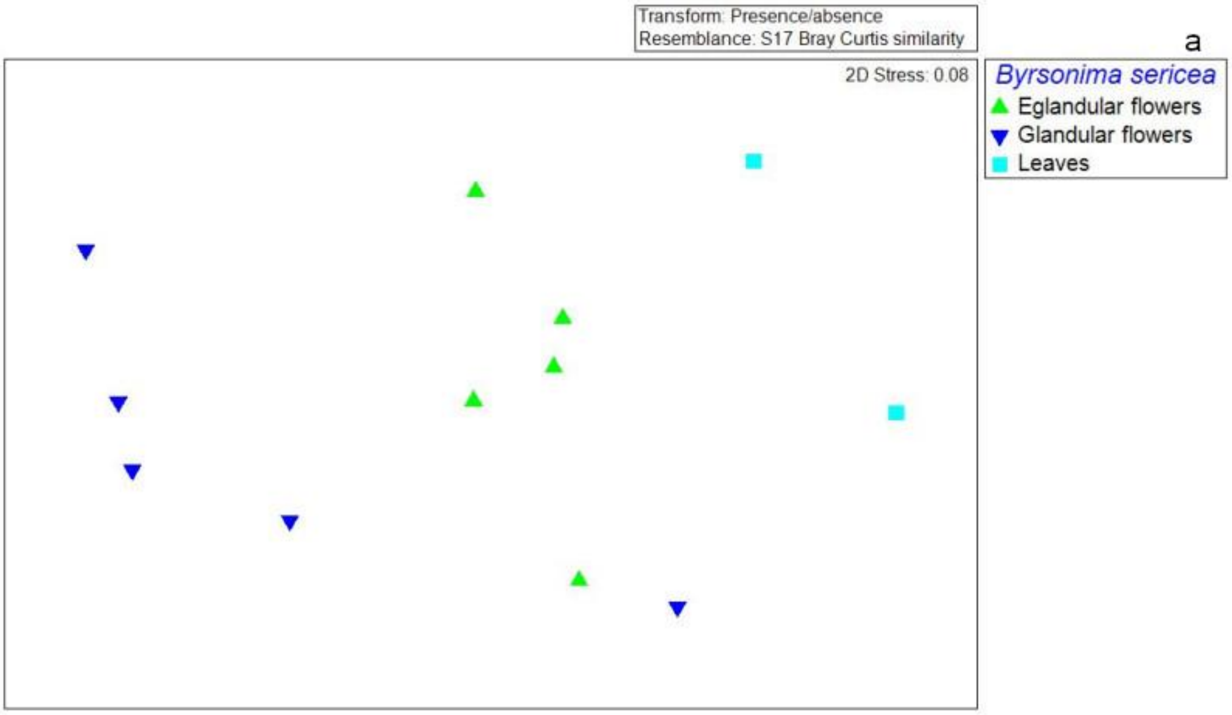
Compounds scents *Byrsonima sericea*	RI	Eglandular flowers	Glandular flowers	Leaves of eglandular flowers	Leaves of glandular flowers
Nonanal	1105	X	X		
Ethylhexanoic acid	1117		X		

Decanal	1205	X		X	
cf. Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl) propyl ester	1364		X		
cf. Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	1377	X	X		
Dodecanal	1409				X
trans-beta-Farnesene	1455		X		
Germacrene D	1488		X		
Tridecanal	1511		X		X
dodecanoic acid	1561		X		
Unknown	1604	X			
Tetradecenal	1613		X		X
Pentadecenal	1715		X		X
Tetradecanoic acid	1759		X		
Octadecane	1800	X			
cf. hexadecenal	1817	X	X		X
cf. hexadecanol	1880		X		
Hexadecanoic acid, methyl ester	1926	X	X		
hexadecanoic acid	1960	X	X		
Unknown	2021		X		
(E,E)-Geranyl linalool	2032		X		
Methyl linoleate	2096		X		
Unknown	2108		X		
Unknown	2161		X		
Unknown	2225		X		
Unknown	2270		X		
Unknown	2291		X		

Unknown	2360	X	X		
Unknown	2386		X		
Unknown	2476		X		
Unknown	2489		X		
Pentacosane	2500	X	X		
Unknown	2531		X		
Unknown	2560		X		
Hexacosane	2600	X			
Heptacosane	2700				
Unknown	2655		X		
Heptacosane	2700	X	X	X	X
Octacosane	2800	X	X	X	X
Unknown	2813	X	X		
Nonacosane	2900	X	X	X	X
Unknown	2930		X		
Unknown	2979	X	X		
Unknown	2985		X		
Unknown	2991		X		
Triacontane	3000	X	X	X	X
Unknown	3017		X		
Unknown	3047	X			
Unknown	3080	X		X	X
cf. Heptacosanol	3083		X		
Henetriacontane	3100	X	X	X	X
Unknown	3129		X		
Unknown	3160			X	
Unknown	3178	X	X		

Unknown	3228		X		
Unknown	3234	X	X		X
Unknown	3245		X		
Unknown	3258	X			
Unknown	3262	X	X		
Unknown	3269	X	X		
Unknown	3279	X	X		
Tritriacontace	3300	X	X		
Unknown	3318	X	X		
Unknown	3324		X		
Unknown	3334	X	X		
Unknown	3338	X	X		
Unknown	3351	X	X		
Unknown	3366	X	X		
Unknown	3369		X		
Unknown	3380	X	X		
Unknown	3381	X	X	X	X
Unknown	3385		X		
Unknown	3410	X	X	X	X
Unknown	3450	X			X
Unknown	3460		X		X
Unknown	3468				X
Unknown	3504	X	X		X
Unknown	3542		X		
Unknown	3571		X		

Fonte: Autor, 2021.



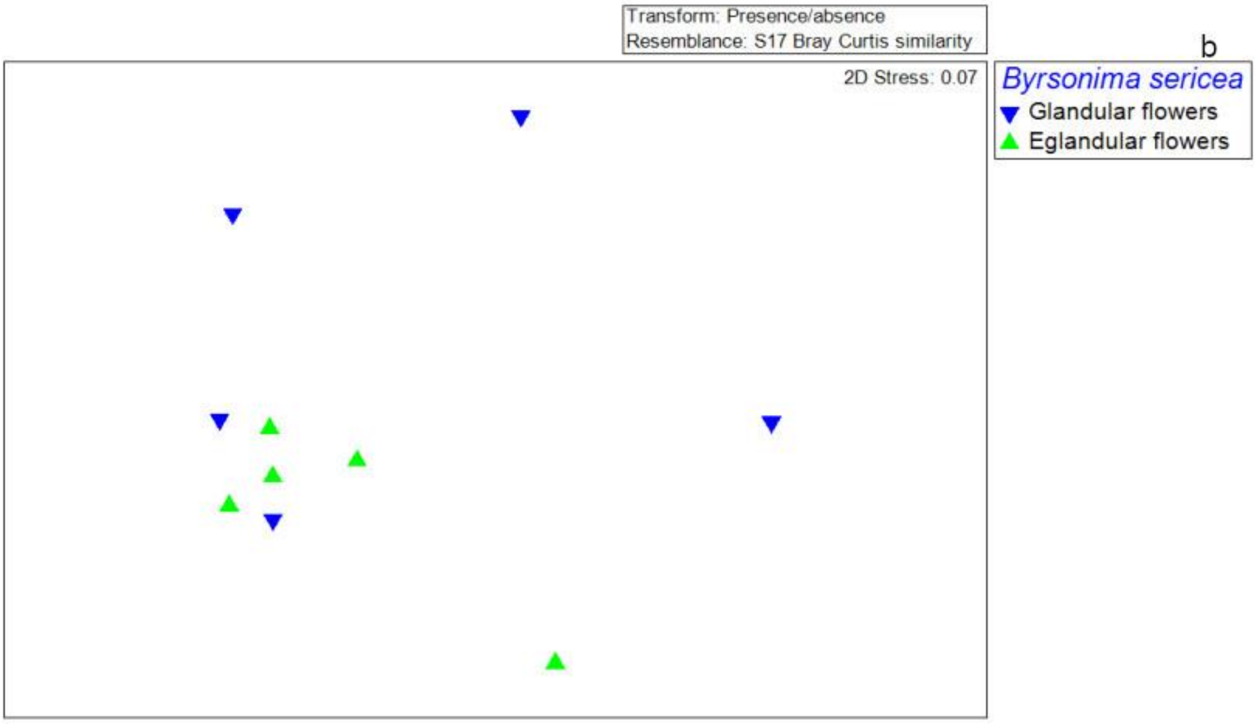


Figure 4 - Multidimensional Scaling (MDS) of: a) samples extract of floral scents and leaves (n=2) scents of glandular (n=5) and eglandular flowers (n=5) of Byrsonima sericea (Malpighiaceae). ANOSIM Global R = 0.612; p = 0.001. b) Samples headspace of floral scents of glandular (n=5)

and eglandular flowers (n=5) of Byrsonima sericea (Malpighiaceae). ANOSIM r = 0.028; p = 0.34. Fonte: Autor, 2021.

Headspace samples - In the floral headspace samples of *B. sericea*, 28 compounds have been identified. The major compounds were (*E*)-β-Ocimene (glandular flowers = 60.68%; eglandular flowers = 64.46%) and β-Caryophyllene (glandular flowers = 15.31%; eglandular flowers = 6.18%) (Tab. 3). The chemical composition of floral volatiles identified in both morphotypes did not differ significantly (ANOSIM, *r* = 0.028; *p* = 0.34) (Fig.4).

TABLE 3. *Scents compounds of glandular and eglandular flowers of* Byrsonima sericea *(Malpighiaceae). RI = Retention index.*

		Glandular flowers		Eglandular flowers	
Compound name	IR	Mean relative amount (%)	Standard deviation	Mean relative amount (%)	Standard deviation
Hexanal*	799	2.10	2.13	2.15	2.07
1-Hexanol*	855	0.87	1.95	2.58	1.75
α-Pinene	929	0.00	0.00	0.23	0.51
β-Myrcene*	988	1.03	1.09	0.87	0.71
α-Terpinene	1013	1.54	1.49	3.13	1.91
(*Z*)-β-Ocimene*	1037	2.86	2.03	2.06	0.96
(*E*)-β-Ocimene*	1047	60.68	11.57	64.46	17.09
(*Z*)-Linalool oxide (furanoid)*	1071	0.12	0.27	0.05	0.11
(*E*)-Linalool oxide (furanoid)*	1086	1.18	2.25	1.19	0.42
Linalool*	1098	2.08	2.71	3.78	2.69
(*E*)-4,8-dimethyl-1,3,7-nonatriene*	1116	4.93	1.59	2.96	1.47
Neo-allo-Ocimene	1142	0.26	0.57	0.11	0.24
(*E*)-Ocimene epoxide*	1142	0.07	0.16	0.07	0.17

Hexyl isobutyrate*	1148	0.00	0.00	0.30	0.50
Hexyl butyrate*	1191	1.16	1.15	1.91	2.68
(*Z*)-3-hexenyl 3-methylbutanoate*	1236	1.16	1.07	2.77	3.06
Hexyl-2-methylbutanoate	1237	0.00	0.00	0.51	0.93
Hexyl isovalerate *	1241	0.27	0.37	0.73	0.89
(3*Z*)-Hexenyl tiglate	1324	1.58	1.73	1.10	1.48
Hexyl tiglate*	1330	0.47	0.74	1.33	1.48
α-Cubebene	1351	0.78	1.04	0.76	0.87
Eugenol*	1359	0.00	0.00	0.10	0.22
Hexyl hexanoate*	1386	0.15	0.35	0.16	0.35
β-cubebene	1392	0.17	0.39	0.00	0.00
β-Elemene	1394	0.24	0.53	0.00	0.00
β-Caryophyllene*	1423	15.31	13.73	6.18	3.25
α-Humulene	1457	0.98	0.98	0.26	0.17
(*E*,*E*)-α-Farnesene*	1508	0.00	0.00	0.23	0.52
Total Ion Current (TIC)		48004326		74937039.6	
Area external standard (splitless=200ng)		44481883.73		44481883.73	
Total amount of scent (ng) split 1:5		215.8376488		336.9328514	
Race split		5		5	
Total scent amount (ng)		1079.188244		1684.664257	
Sampling time (min)		60		60	
Aumont of scent per minute		17.98647073		28.07773761	

Fonte: Auto. 2021.

3.4. DISCUSSION

The loss or reduction of floral oil glands within neotropical species of Malpighiaceae is expected to be an adaptation associated with the energetic costs involved in the synthesis of fatty acids (Vogel, 1990). However, the loss of oil offering should contribute to a higher exploitation of pollen by the bees, and thus pollinator deception via floral cues should be an advantageous strategy in these systems. In dimorphic populations of *B. sericea*, the absence of floral oil production should provide higher resource allocation for both male and female functions if floral cues related to the attraction and visitation by oil bees are maintained in eglandular individuals. Nevertheless, olfactory cues involved in oil-seeking bee attraction may be produced in association with oil synthesis (Schäffer *et al.*, 2015). In this context, our results indicate that both morphs share similarities in floral cues and that differences in floral volatiles synthetized via lipid routes may not be perceived by the bees at long distances. Therefore, oil-seeking bees such as *Epicharis* can be deceived by eglandular individuals, which potentially benefit from their oil-collecting movements on their flowers. However, the similarities in floral cues and absence of energetic costs associated with oil production may not provide higher female reproductive success to the eglandular morph.

Both morphs were UV-reflective as most bee-pollinated flowers (Chittka *et al.*, 1994), and shared similar reflectance properties as expected, since color-based polymorphisms have not been documented for Malpighiaceae. Intraspecific variation in floral color has been only observed at the flower level within family; *i.e.*, color shift during anthesis (*e.g.*, Anderson, 1979; Melo *et al.*, 2018). Therefore, the visiting bees might not discriminate the morphs of *B. sericea* based on visual cues only. The reflectance of the oil glands was not measured in our study due to the option of measuring only the frontal floral parts. Nevertheless, there are evidences from other studies indicating that oil bees do not perceive the absence of oil glands in the flowers before landing. This is supported by the fact that bees briefly display the stereotyped oil collecting behavior even after landing on an eglandular flower (Ferreira and Torezan-Silingardi, 2013; Carneiro *et al.*, 2019).

In the scent samples collected through dynamic headspace methods, we found that the floral volatiles emitted by glandular and eglandular flowers are also similar. The major compounds identified in the flowers through this method were (*E*)-β-Ocimene and β-Caryophyllene, which are not specific to oil bee attraction. Instead, (*E*)-β-Ocimene is quite common as constituent of scents of both flowers and leaves of angiosperms, and seem to play an important role in pollinator attraction and defense against herbivores in generalist and specialist plant species (Farré-Armengol *et al.*, 2017). β-Caryophyllene is also common and has been reported as an important volatile against bacterial growth (Huang *et al.*, 2012). Dynamic headspace is an appropriate method for sampling volatile organic compounds, but it fails to sample low-volatile compounds. Volatile organic compounds are transported in the form of plumes by the wind and consequently can be perceived by the bees at long distances (Milet-Pinheiro *et al.*, 2020). In contrast, heavier compounds such as fatty acid derivates present a lower volatility and are usually detected in solvent extracts of flowers (Schäffler *et al.*, 2015).

In the flower extracts, we found that both morphotypes have typical fatty acids derivates from plant tissues and commonly involved in waterproofing (Schiestl *et al*., 2000). From the glandular flowers, we additionally identified other compounds derivate from fatty acids secreted through the oil glands. The bees may only perceive the absence of these additional compounds after approaching or landing on the eglandular flowers. The attempt of the oil bees to collect oil when visiting the eglandular morphotype is good be evidence of their inability to discriminate between flowers of different morphs. We believe that differences in floral compounds between the morphotypes of *B. sericea* can only be found through flower extracts, indicating that the bees can only detect floral oil derivate volatiles after landing on flowers, i.e. at short distances. Therefore, the bees should not be able to discriminate floral scent emissions from both morphs at some distance.

Similarities in visual and olfactory cues between floral morphs indicates that the eglandular morph can deceive the pollinators at long distance. This may explain the fact that the individuals in the studied populations were similar in flower-visiting assemblage regardless their floral morphotype. We also detected no differences in the female reproductive success (measured as fruit set) between glandular and eglandular morphs as also found in previous studies (Teixeira and

Machado, 2000; Rosa *et al*., 2007). This result indicates that the loss of oil production does not lead to a higher resource allocation at least for the measured female fitness component. Since oil production requires high energetic costs, the absence of lipid synthetic routes is expected to be correlated with a positive impact on seeds. Perhaps, the costs involved in the development of the three-seeded malpigh fruits may be minimum to be negatively influenced by the synthesis of fatty acids, and other female components and fruit traits should be more prospective for evaluation by further studies. However, the impacts of the loss of oil production seems to be higher considering the plant male function as indicated by Teixeira and Machado (2000). These authors found that eglandular flowers produce 20% more pollen grains compared to glandular flowers, suggesting resource allocation towards the enhancement of pollen delivery. Nevertheless, the loss of pollen by bees' consumption should also be higher, unless deceptive oil visits on eglandular flowers are proportional compared to those oil visits on glandular flowers.

The ecological and evolutionary outcomes involved in the reduction or absence of oil secretion within some neotropical clades of Malpighiaceae have been poorly understood. In the case of the dimorphism found in *B. sericea*, which is featured by the presence of glandular and eglandular individuals within the same populations, the loss of oil production is not associated with changes in floral cues (visual and olfactory) used in pollinator attraction. This may cause a deceptive mechanism that triggers the oil-collecting behavior of bees even in the absence of oil glands. An increased female reproductive success was not observed in the eglandular morphotype, suggesting at least that the energetic costs associated with oil production are not allocated for fruit set. Further studies should assess the impact of floral oil loss in the male fitness, which seems to be most influenced by energetic tradeoffs. It would be also interesting to evaluate whether bees seeking for oil are more efficient in pollen pickup and deposition than bees seeking for pollen, and understand whether the deceptive mechanism associated with similarities in floral cues between glandular and eglandular morphs are sufficient to maintain the deceit of oil bees, while avoiding changes in bee preferences at the same time.

3.5 ACKNOWNLEDGEMENTS

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3.6 REFERENCES

Aliscioni SS, Gotelli M, Torretta JP. 2018. Structure of the stigma and style of *Callaeum psilophyllum* (Malpighiaceae) and its relation with potential pollinators. *Protoplasma* 255: 1433–1442.

Alves-Dos-Santos I, Machado IC, Gaglianone MC. 2007. História natural das abelhas coletoras de óleo. *Oecologia Brasiliensis* 11:544–557.

Anderson WR. 1979. Floral conservatism in neotropical Malpighiaceae. *Biotropica* 11: 219.

Anderson WR. 1990. The origin of the Malpighiaceae-The evidence from morphology. In: Memoirs of New York Botanical Garden. 64: 210 – 224.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67: 1–48

Burger H, Ayasse M, Häberlein CM, Schulz S, Dötterl S. 2010. Echium and Pontechium specific floral cues for host-plant recognition by the oligolectic bee *Hoplitis adunca*. *South African Journal of Botany* 76:788–795.

Campbell DR, Bischoff M, Lord JM, Robertson AW. 2012. Where have all the blue flowers gone: pollinator responses and selection on flower color in New Zealand *Wahlenbergia albomarginata*. *Journal of Evolutionary Biology* 25: 352-364.

Carneiro LT, André CBDS, Takahasi A, Alves-dos-Santos I. 2019. Interactions between oil-collecting bees and *Krameria grandiflora* (Krameriaceae) with emphasis on the role of specialized floral traits in the mutual fit. *Arthropod-Plant Interactions* 13: 213–226.

Chittka L. 1992. The color hexagon – a chromaticity diagram based on photoreceptor excitations as a generalized representation of color opponency. *Journal of Comparative Physiology A: Sensory, Neural and Behavioral Physiology* 170: 533–543.

Chittka L, Shmida A, Troje N, Menzel R. 1994. Ultraviolet as a component of flower reflections, and the color-perception of Hymenoptera. *Vision Research* 34:1489–1508.

Costa CBN, Costa JAS, Ramalho M. 2006. Biologia reprodutiva de espécies simpátricas de Malpighiaceae em dunas costeiras da Bahia, Brasil. *Revista Brasileira de Botânica* 29: 103-11.

Cruden RW. 2000. Pollen grains: Why so many? *Plant Systematics and Evolution* 222(1-4): 143–165.

Climate-data.org. 2019. Dados climáticos para as cidades mundiais. Available: https://pt.climate-data.org/america-do-sul/brasil/pernambuco/camaragibe33802/?fbclid=IwAR2jU8CzGnuX0kyuhIptmOs0aRzTaZKsZh9uwiSo9BDCt1c8-GEEWdX0w. Accessed: Jan 2019.

Davis CC. 2002. *Madagasikaria* (Malpighiaceae): a new genus from Madagascar with implications for floral evolution in Malpighiaceae. *American Journal of Botany* 89(4): 699 -706.

Davis CC, Anderson WR. 2010. A complete generic phylogeny of Malpighiaceae inferred from nucleotide sequence data and morphology. *American Journal of Botany* 97(12): 2031-2048.

Davis CC, Schaefer H, Xi Z, Baum DA, Donoghue MJ, Harmon LJ. 2014. Long-term morphological stasis maintained by a plant-pollinator mutualism. *Proceedings of the National Academy of Sciences* 111(16): 5914–5919.

Dötterl S, Milchreit K, Schäffler I. 2011. Behavioural plasticity and sex differences in host finding of a specialized bee species. *Journal of Comparative Physiology A* 197(12): 1119- 1126.

Farré-Armengol G, Filella I, Llusià J, Peñuelas J. 2017. β-Ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. *Molecules* 22: 1148.

Ferreira CA, Torezan-Silingardi HM. 2013. Implications of the floral herbivory on Malpighiaceae plant fitness: Visual aspect of the flower affects the attractiveness to pollinators. *Sociobiology* 60: 323–328.

Flora Do Brasil. 2019. *Byrsonima in Flora do Brasil 2020 em construção.* Jardim Botânico do Rio de Janeiro. Available: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB8845>. Accessed: Fev 2019.

Huang M, Sanchez-Moreiras AM, Abel C, *et al*. 2012. The major volatile organic compound emitted from *Arabidopsis thaliana* flowers, the sesquiterpene (E)-β-caryophyllene, is a defense against a bacterial pathogen. *New Phytologist* 193: 997–1008.

Lenth RV. 2016. Least-Squares Means: The *R* Package lsmeans. *Journal of Statistical Software* 69:1–33.

Lima PJ, Heckendorff WD. 1985. *Climatologia. In: Governo do Estado da Paraíba. Atlas geográfico do Estado da Paraíba*. Universidade Federal da Paraíba, João Pessoa.

Lunau K. 1992. Innate recognition of flowers by bumble bees: orientation of antennae to visual stamen signals. *Canadian Journal of Zoology* 70: 2139-2144.

Machado IC, Vogel S, Lopes AV. 2002. Pollination of *Angelonia cornigera* Hook. (Scrophulariaceae) by long-legged oil-collecting bees in NE Brazil. *Plant Biology* 4: 352-359.

Melo BT, Mota T, Schilindwein C, Antonini Y, Oliveira R. 2018. Floral colour change in *Byrsonima variabilis* (Malpighiaceae) as a visual cue for pollen but not oil foraging by oil-collecting bees. *The Science of Nature* 105:46.

Milet-Pinheiro P, Ayasse M, Dötterl S. 2015. Visual and olfactory floral cues of *Campanula* (Campanulaceae) and their significance for host recognition by an oligolectic pollinator. *PLoS ONE* 10(6).

Milet-Pinheiro P, Domingos-Melo A, Oliveira-Júnior JB, *et al*. 2020. A semivolatile floral scents marks the shift to a novel pollination system in Bromeliads. *Current Biology* 31, 860-868.

Papadopulos AST, Powell MP, Pupulin F *et al*. 2013. Convergent evolution of floral signals underlies the success of Neotropical orchids. *Proceedings of the Royal Society B: Biological Sciences* 280, 20130960.

Plants of the World online. 2019. Available: http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:556185-1#distribution-map. Accessed: May 2019.

Policha T, Davis A, Barnadas M, Dentinger BTM, Raguso RA, Roy BA. 2016. Disentangling visual and olfactory signals in mushroom-mimicking *Dracula orchids* using realistic three-dimensional printed flowers. *New Phytologist,* 210(3): 1058 -1071.

Possobom CCF, Machado SR. 2017. Elaiophores: their taxonomic distribution, morphology and functions. *Acta Botanica Brasilica* 31(3): 503-524.

R Core Team. 2019. *R: A language and environment for statistical computing.* Vienna: R Foundation for Statistical Computing.

Renner SS, Schaefer H. 2010. The evolution and loss of oil-offering flowers: new insights from dated phylogenies for plants and bees. *Philosophical Transactions of the Royal Society B* 365: 423-435.

Rosa JF, Ramalho M. 2007. Sucesso reprodutivo de *Byrsonima sericea* DC. (Malpighiaceae) e diversidade de abelhas Centridini (Apidae). *Revista Brasileira de Biociências* 5 (1):168-170.

Sazima M, Sazima I. 1989. Oil-gathering bees visit flowers of eglandular morphs of the oil-producing Malpighiaceae. *Botanica Acta* 102: 106-111.

Schäffler I, Balao F, Dötterl S. 2012. Floral and vegetative cues in oil-secreting and non-oil-secreting *Lysimachia* species. *Annals of Botany* 110: 125-138.

Schäffler I, Steiner KE, Haid M, *et al*. 2015. Diacetin, a reliable cue and private communication channel in a specialized pollination system. *Scientific Reports* 5: 12779.

Schiestl FP, Ayasse M, Paulus HF, *et al*. 2000. Sex pheromone mimicry in the early spider orchid (*Ophrys sphegodes*): patterns of hydrocarbons as the key mechanism for pollination by sexual deception. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology* 186(6): 567–574.

Sigrist MR, Sazima M. 2004. Pollination and reproductive biology of twelve species of neotropical Malpighiaceae: stigma morphology and its implications for the breeding system. *Annals of Botany* 94: 33–41.

Teixeira LM, Machado IC. 2000. Sistemas de polinização e reprodução de *Byrsonima sericea* DC (Malpighiaceae). *Acta Botanica Brasilica* 14: 347-357.

Vogel S. 1969. Flowers offering fatty oil instead of nectar. In: XI Proc. Int. Bot. Cong. Abstracts. Seattle. 229.

Vogel S. 1974. Ölblumen und ölsammelnde Bienen. Tropische und Subtropische Pflanzenwelt 7: 285-547.

Vogel S. 1990. History of the Malpighiaceae in the light of pollination ecology. *Memoirs of the New York Botanical Garden* 55: 130-142.

Vogel S, Machado IC. 1991. Pollination of four sympatric species of *Angelonia* (Scrophulariaceae) by oil-collecting bees in NE Brazil. *Plant Systematics and Evolution* 178: 153-178.

Zhang W, Kramer EM, Davis CC. 2010. Floral symmetry genes and the origin and maintenance of zygomorphy in a plant-pollinator mutualism. *Proceedings of the National Academy of Sciences* 107(14): 6388–6393.

Zhang W, Kramer EM, Davis CC. 2012. Similar genetic mechanisms underlie the parallel evolution of floral phenotypes. *PLoS One*, 7(4): e36033.

Zhang W, Steinmann VW, Nikolov L, Kramer EM, Davis CC. 2013. Divergent genetic mechanisms underlie reversals to radial floral symmetry from diverse zygomorphic flowered ancestors. *Frontiers in Plant Science* 4.

3.7 SUPPLEMENTARY DATA

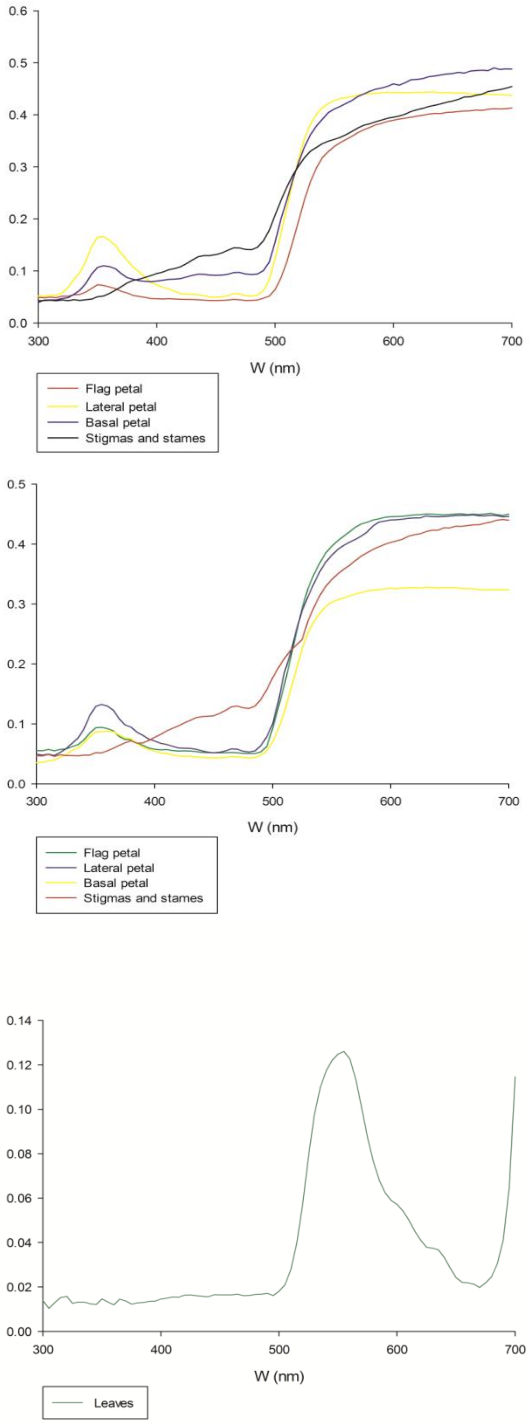

Figure S1. *Mean spectral reflection of (A) glandular, (B) eglandular flowers and (C) leaves of* Byrsonima sericea *(Mapighiaceae)*. Fonte: Autor, 2021.

TABLE S1. Posteriori pairwise comparisons of morphs of *Byrsonima sericea*. Analysis of similarity (ANOSIM) of scents of glandular and eglandular flowers, R = 0.612; p = 0.001

Groups	R	P
Eglandular and glandular flowers	0.55	0.016
Eglandular flowers and leaves	0.81	0.048
Glandular flowers and leaves	0.68	0.048

Fonte: Autor, 2021.

4. CONCLUSÃO

As espécies neotropicais de flores de óleo estudadas das famílias: Malpighiaceae, Krameriaceae, Plantaginaceae e Iridaceae mostram uma grande diversidade de padrões de cores e/ou odores florais, sendo a composição dos odores florais espécie específica e com pouca/nenhuma relação ao gênero ou família da espécie de flor de óleo analisada. Essa diversidade de padrões visuais e olfativos pode refletir na diferença de composição das espécies visitantes de abelhas coletoras de óleo.

A mudança de cor das flores durante a antese é evidenciada no modelo de visão das abelhas, para *Banisteriopsis muricata* a pétala estandarte é a estrutura diferenciada, já em *Byrsonima gardineriana*, as diferenças de diferentes estruturas parecem ser um sinal de honestidade floral, indicando as abelhas à presença do recurso óleo.

O padrão de cor e o perfil de voláteis dos odores florais são semelhantes nos dois morfos de *Byrsonima sericea*, mas existem compostos mais pesados, apenas identificados nos extratos de odores florais das flores com glândulas. Não há diferença na composição dos visitantes florais e não ocorre diferença no sucesso reprodutivo dos dois morfos. As abelhas coletoras de óleo tentam coletar este recurso nas flores sem óleo, sem sucesso, acabam realizando a coleta de pólen. Portanto, os sinais visuais e olfativos à longa distância parecem enganar os polinizadores e garantir o sucesso reprodutivo também do morfo sem glândulas de óleo.

ANEXO - Primeira página nas normas da revista *Annals of Botany*

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Introduction

Scope of the jornal

Annals of Botany is published for the Annals of Botany Company by Oxford University Press. Experimental, theoretical and applied papers on all aspects of plant science are welcome. To merit publication in *Annals of Botany*, contributions should be substantial, written in clear English and combine originality of content with potential general interest. The manuscript or its essential content must not have been published or be under consideration for publication in other journals, but may be published in a thesis or as an abstract, and we encourage deposit on preprint servers such as BioRxiv. Submission of manuscripts that report small incremental advances or are of geographically local interest only is discouraged unless the implications of the findings are wide-reaching. Agronomic papers are expected to contain a substantial amount of basic plant biology. In general, a paper is unlikely to be accepted unless the referees and editors involved in its evaluation are enthusiastic about the science. The Covering Letter is an essential part of all submissions. It should include an ~ 60 word summary of the scientific strengths of the paper that the author(s) believe qualify it for consideration by *Annals of Botany*.

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