

UNIVERSIDADE DE SÃO PAULO  
FACULDADE DE FILOSOFIA, CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO  
PROGRAMA DE PÓS-GRADUAÇÃO EM ENTOMOLOGIA

**“*Xylocopa frontalis* Oliver and *Xylocopa grisescens* Lepeletier (Apidae, Xylocopini) in  
Brazilian savanna: pollination services in passion fruit (*Passiflora edulis* f. *flavicarpa*  
Degener), nesting behaviors and population dynamics.”**

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serviços de polinização no maracujá-amarelo (*Passiflora edulis* f. *flavicarpa* Degener),  
comportamentos de nidificação e dinâmica populacional.”

Ana Luisa de Sousa e Castro Melo

Tese apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, como parte das exigências para obtenção do título de Doutor em Ciências, obtido no Programa de Pós-Graduação em Entomologia.

Ribeirão Preto - SP

2023

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VERSÃO CORRIGIDA

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Orientadora: Profa. Dra. Solange Cristina Augusto

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Castro-Melo, Ana Luisa Sousa

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*A minha mãe, Rosana e minha irmã, Déborah.  
Nada seria possível sem vocês ao meu lado.*

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## RESUMO

Castro-Melo, A. L. S. “*Xylocopa frontalis* Oliver e *Xylocopa grisescens* Lepeletier (Apidae, Xylocopini) no cerrado: serviços de polinização no maracujá-amarelo (*Passiflora edulis* f. *flavicarpa* Degener), comportamentos de nidificação e dinâmica populacional.” Tese - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, 2023, 109 páginas.

Os serviços ecossistêmicos são benefícios que o ambiente oferece para seu próprio equilíbrio e que resultam em benefícios para os seres humanos. No Brasil, a polinização relacionada à produção agrícola é estimada em US\$ 12 bilhões anuais, sendo o maracujá uma cultura dependente essencial de polinização cruzada, o que dificulta a produtividade e aumenta os custos de produção. As abelhas do gênero *Xylocopa* são os principais polinizadores do maracujá-amarelo com variações entre espécies de acordo com a região de plantio. Esta tese teve como objetivo geral analisar os serviços de polinização dos visitantes florais em cultivos de maracujá-amarelo e sua relação com o uso do solo do entorno no cerrado. Além disso, considerando apenas os polinizadores *X. frontalis* e *X. grisescens*, avaliamos a dinâmica populacional e os comportamentos de nidificação em áreas de criação já estabelecidas há mais de uma década, e iniciamos populações de *X. frontalis* em áreas naturais e de cultivos, com o objetivo de contribuir para o manejo dessas abelhas, visando a conservação e o uso em polinização assistida. No capítulo 1, verificamos que o uso intenso do solo no entorno dos cultivos de maracujá afeta negativamente a presença do polinizador efetivo, *X. frontalis*, e a produtividade do cultivo. Áreas de floresta estão relacionadas a maior abundância de *Xylocopa frontalis* e produtividade do maracujá, e áreas agrícolas relacionadas a maior abundância de *Apis mellifera*. Também observamos que áreas com baixo déficit polínico exibiram produção de frutos até cinco vezes maior e apresentaram maior quantidade de polpa nos frutos. No segundo capítulo, observou-se que *X. frontalis* e *X. grisescens* têm comportamentos diferentes de nidificação ao longo do tempo e picos reprodutivos em diferentes períodos do ano. No capítulo 3, observou-se que as populações de *X. frontalis* iniciadas nos abrigos em áreas naturais de cerrado *sensu stricto* foram mais longevas e com maior número de abelhas.

**Palavras-chave:** mamangava, ninhos-armadilha, maracujá-amarelo, manejo de polinizadores.

## ABSTRACT

Castro-Melo, A. L. S. “*Xylocopa frontalis* Oliver and *Xylocopa grisescens* Lepageletier (Apidae, Xylocopini) in Brazilian savanna: pollination services in passion fruit (*Passiflora edulis* f. *flavicarpa* Degener), nesting behaviors and population dynamics.” Tese - Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo, 2023, 109 páginas.

Ecosystem services are benefits that the environment offers for its own balance and that result in benefits for humans. In Brazil, agricultural-related pollination is estimated at US\$12 billion annually, with yellow passion fruit being an essential cross-pollination dependent crop, which hold back productivity and increases production costs. *Xylocopa* bees are the main pollinators of yellow passion fruit with variations between species according to the region. The general objective of this thesis was to analyze the pollination services of floral visitors in yellow passion fruit crops and their relationship with the surrounding land use in the Brazilian savanna. In addition, considering only the pollinators, *X. frontalis* and *X. grisescens*, we evaluated the population dynamics and nesting behaviors in breeding areas already established for more than a decade, and initiated *X. frontalis* populations in natural and crop areas throughout the studied region, with the aim of contributing to the management of these bees for conservation and use in assisted pollination. In chapter 1, we found that the intense land use around passion fruit crops negatively affects the presence of the effective pollinator, *X. frontalis*, and the productivity of the crop. Pasture areas are relevant to higher passion fruit productivity, forest areas are related to higher abundance of *Xylocopa frontalis*, and agricultural areas are related to higher abundance of *Apis mellifera*. We also observed that areas with low pollination deficit (LPD) exhibited fruit set up to five times greater and presented a higher amount of pulp in the fruits. In chapter 2, we observed that *X. frontalis* and *X. grisescens* have different nesting behaviors over time and reproductive peaks in different periods of the year. In chapter 3, we observed that the initiated *X. frontalis* populations were longer-lived and had a greater number of bees in the shelters in natural Brazilian savanna areas (cerrado *sensu stricto*). From the establishment of two larger populations, we recorded four different natural enemies and complex intraspecific behaviors.

**Keywords:** Carpenter bees, trap-nests, passion fruit, pollinator management.

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## INTRODUÇÃO GERAL

Serviços ecossistêmicos são benefícios que o ambiente desempenha para seu próprio equilíbrio e que resultam em benefícios para os seres humanos. Eles são divididos em serviços de provisão, como o fornecimento de alimentos, água e matérias-primas; serviços de regulação, como o controle do clima e a purificação da água; e serviços culturais, como recreação e enriquecimento espiritual (Hueting *et al.*, 1998; Millennium Ecosystem Assessment, 2003; FAO, 2007). Em um conceito mais amplo e menos antropocêntrico, as funções ecossistêmicas abrangem serviços ecossistêmicos independentemente de seu uso, demanda ou valoração social, ignorando se essas interações beneficiam ou não os seres humanos (Prado, 2014).

O valor econômico dos serviços de polinização é estimado entre US\$ 235 bilhões e US\$ 577 bilhões globalmente (IPBES, 2016). No Brasil, o valor anual da polinização relacionada à produção agrícola é estimado em US\$ 12 bilhões (Giannini *et al.*, 2015), e difere entre espécies de plantas e cultivos agrícolas de acordo com a dependência de polinização, que pode ser ausente, pouca, moderada, alta ou essencial (Klein *et al.*, 2007; Giannini *et al.*, 2015).

A maioria das plantas, tanto cultivadas quanto nativas, depende de polinizadores animais para a reprodução em pelo menos um nível de dependência (Klein *et al.*, 2007, Ollerton *et al.*, 2011; Roubik, 2018). A polinização biótica é um importante serviço ecossistêmico, fundamental para a reprodução da maioria das espécies vegetais, incluindo aquelas cultivadas para a produção de alimentos. Estima-se que 35% da produção agrícola seja de cultivos com alguma dependência da polinização biótica para produção de frutos (Klein *et al.*, 2007). Abóbora, acerola, cajá, castanha de caju, castanha-do-brasil, cupuaçu, maçã, maracujá, melancia, melão e tangerina são algumas das muitas culturas brasileiras importantes que dependem essencialmente da polinização para a produção de frutas e sementes, com um aumento na produção entre 90% e 100% na presença de polinizadores (Wolowski *et al.*, 2019).

Cultivos agrícolas como a acerola (*Malpighia emarginata*) e maracujá amarelo (*Passiflora edulis*) requerem a visita de abelhas especializadas de médio a

grande porte para realizar visitas florais que proporcionam a formação de frutas e sementes (Cavalcante *et al.*, 2012; Cobra *et al.*, 2015; Freitas *et al.*, 1999; Gaglianone *et al.*, 2010; Junqueira; Augusto, 2017; Vilhena *et al.*, 2012). Além disso, algumas espécies botânicas são autoincompatíveis, o que significa que suas flores não são fertilizadas pelo próprio pólen, necessitando de polinização cruzada para produzir frutos, classificando essas plantas como essencialmente dependentes de polinização (Klein *et al.*, 2007).

O maracujá é uma planta trepadeira ou liana que possui gavinhas para se prender a outras plantas ou estruturas, pertencente à família *Passifloraceae*. O centro de diversidade do maracujá está principalmente localizado na região neotropical, representando aproximadamente 95% de todas as espécies de *Passiflora* na América do Sul, com algumas espécies também encontradas no Velho Mundo, Sudeste Asiático, Austrália e Ilhas do Pacífico (Yockteng *et al.*, 2011).

De acordo com a Organização das Nações Unidas para Agricultura e Alimentação (FAO), os principais produtores globais de maracujá são Brasil, Equador e Colômbia. Entre as variedades cultivadas, *Passiflora edulis f. flavicarpa*, também conhecido como maracujá amarelo, se destaca e é responsável por quase toda a produção brasileira. Sua fruta é comestível e de alto valor comercial, sendo que no Brasil, essa cultura é de grande importância econômica e social, sendo cultivada por pequenos agricultores que usam as frutas como fonte complementar de renda, vendendo-as frescas ou para indústrias de processamento de suco de frutas (Yamamoto; Barbosa; Oliveira, 2010).

Entre 2017 e 2021, a produção de maracujá teve expressivo crescimento no Brasil, com um aumento de mais de 50% no valor da produção nacional, passando de menos de 855 milhões de reais para mais de um bilhão e meio de reais anualmente. Os maiores produtores no Brasil são os estados da Bahia, Ceará, Santa Catarina e Minas Gerais, respectivamente. Embora a Bahia tenha a maior área plantada (16.816 hectares), sua produtividade (12.339 kg por hectare) é menor do que a média nacional de 15.259 kg por hectare e menor do que os valores observados para os outros principais estados produtores: Ceará

(24.043 kg por hectare), Santa Catarina (25.161 kg por hectare) e Minas Gerais (17.146 kg por hectare) (IBGE 2021).

As abelhas dos gêneros *Xylocopa*, e algumas espécies de *Centris*, *Epicharis*, *Eulaema* e *Bombus* foram registradas como polinizadoras de maracujá no Brasil, sendo que as espécies de *Xylocopa* são as polinizadoras naturais mais eficientes do cultivo (Corbet; Willmer, 1980; Sazima; Sazima, 1989; Camillo, 2003, Junqueira; Hogendoorn; Augusto, 2012; Pereira; Garófalo, 2010; Yamamoto *et al.*, 2012; Oliveira *et al.*, 2014; Junqueira; Augusto, 2017) devido ao seu grande tamanho e comportamento durante a coleta de néctar das flores (Camillo; Garófalo; Muccillo, 1986). Essas abelhas são grandes e fortes o suficiente para superar a barreira membranosa presente nas câmaras de néctar e podem passar minutos coletando néctar, muitas vezes girando em torno do andrógino, rodeando a flor abaixo das anteras em um comportamento de rotação. À medida que se movem ao redor da câmara de néctar, depositam parte do pólen coletado de suas visitas anteriores no estigma da flor. Quando essas visitas coincidem com o alinhamento dos órgãos reprodutivos, a chance de polinização efetiva aumenta, mesmo com poucas visitas (Silva *et al.*, 2014). Por outro lado, abelhas pequenas como *Apis mellifera* e *Trigona spinipes*, categorizadas como pilhadoras, cortam a membrana da câmara de néctar para acessar o néctar, danificando a flor e prejudicando a produção (Oliveira *et al.*, 2014).

As espécies do gênero *Xylocopa* são abelhas de grande porte e parentes próximos das abelhas corbiculadas sociais (Bossert *et al.*, 2019). As abelhas carpinteiras grandes pertencem exclusivamente ao gênero *Xylocopa* dentro da tribo Xylocopini (Bossert *et al.*, 2019). Elas apresentam uma distribuição predominante em regiões tropicais e subtropicais, mas também podem ser encontradas em áreas temperadas, embora sejam menos comuns nessas regiões (Hurd; Moure, 1963; He; Zhu, 2018).

A subfamília Xylocopinae é considerada a mais basal das linhagens dentro de Apidae, com mais de 700 espécies distribuídas em quatro tribos pelo mundo: Allodapini, Ceratinini, Manuelliini e Xylocopini (Michener, 2007). Essas abelhas são altamente plásticas e importantes modelos para estudos dos estágios iniciais e evolução da sociabilidade, bem como dos comportamentos e história natural

das espécies relacionados a essas variações (Rehan; Leys; Schwarz, 2012). *Xylocopa* é um gênero de abelha cujas espécies nidificam acima do solo, representando aproximadamente 30% de todas as espécies de abelhas (Vaughan *et al.*, 2014). Conhecidas por seu grande tamanho e alta capacidade de voo, essas abelhas utilizam uma vasta área de forrageamento, exibem constância floral e se envolvem em comportamentos de zumbido (King; Buchmann, 2003), permitindo-lhes coletar eficientemente recursos limitados do ambiente. Essa notável adaptação às restrições de recursos, combinada com sua longa vida útil, gerações sobrepostas e cuidado parental, posicionam essas abelhas como um modelo de estudo intrigante para investigar sociedades de espécies de abelhas mais basais (Buchmann; Minckley, 2019)

A principal característica dos ninhos de *Xylocopa* é a presença de partições de serragem entre as células de cria, dispostas linearmente nas galerias escavadas em troncos ou galhos de madeira morta e de textura macia (Rehan; Leys; Schwarz, 2012). Essas partições são compactadas e misturadas com saliva pelas fêmeas para proteger a prole contra desidratação ou umidade excessiva (Gerling; Velthuis; Hefetz, 1989). Cada célula de cria é individualizada e contém uma massa em forma de sino de pólen e um ovo (Lucia *et al.*, 2017; Marchi; Melo, 2010; Pereira; Garófalo, 2010).

As espécies de *Xylocopa*, além dos substratos de madeira naturalmente utilizados, podem também usar diferentes tipos de ninhos-armadilha que são disponibilizados em área pré-estabelecidas para a atração de fêmeas nidificantes. O uso de ninhos-armadilha, permitiu a descrição dos hábitos de nidificação de algumas espécies como *Xylocopa suspecta*, *Xylocopa frontalis*, *Xylocopa grisescens*, *Xylocopa cearencis* e *Xylocopa ordinaria* (Bernardino; Gaglianone, 2013; Camillo; Garófalo, 1989; Camillo; Garófalo, 1982; Camillo; Garófalo; Muccillo, 1986; Chaves-Alves *et al.*, 2011; Marchi; Melo, 2010; Pedroso *et al.*, 2021; Pereira; Garófalo, 2010; Souza *et al.*, 2021).

No Brasil, o fornecimento de ninhos-armadilha em gomos de bambu é amplamente utilizado para estudar a biologia e nidificação de abelhas não-sociais, vespas e seus inimigos naturais (Gazola; Garófalo, 2009). Esses estudos fornecem informações sobre a ocorrência de espécies e a qualidade dos

ambientes naturais e urbanos em que elas estão inseridas, além de entender os processos de fragmentação e urbanização (Rocha-Filho *et al.*, 2021)

Embora o manejo de polinizadores seja uma prática globalmente implementada para aumentar a produção de frutos, o manejo e/ou incremento de polinizadores não-sociais é um grande desafio, principalmente pela biologia e densidade de indivíduos muito menor que aqueles de ninhos sociais. No entanto, um dos poucos exemplos de aplicação deste manejo para incremento de produção é o caso manejo de *X. frontalis* e *X. grisescens* em cultivos de maracujá-amarelo (Junqueira; Augusto, 2017) embora a criação dessas abelhas ainda não seja efetivamente controlada, muito menos comercial.

As abelhas do gênero *Xylocopa* possuem grande raio de voo e altas taxas de dispersão (Pedroso *et al.*, 2021). A ausência de espécies de plantas silvestres como fonte de pólen e néctar e a falta de locais adequados para nidificação, devido à diminuição da cobertura vegetal, vêm contribuindo para a redução das populações de abelhas em áreas de cultivos agrícolas (Aizen *et al.*, 2019; Goulson *et al.*, 2015) e provavelmente levando ao deslocamento dessas populações para áreas ambientalmente mais estáveis (Bezerra *et al.*, 2019)

O Cerrado brasileiro é um bioma com alta diversidade e endemismo (Myers *et al.*, 2000), mas é um dos mais afetados por processos antropogênicos no Brasil, apresentando apenas cerca de 20% de sua vegetação nativa preservada (Colli; Vieira; Dianese, 2020). No entanto, estudos mostram que há plantas com flores durante todo o ano, com pico de florescimento no final da estação seca, o que favorece a manutenção das comunidades de polinizadores ao longo do ano e fornece os recursos necessários para manutenção de ciclos reprodutivos múltiplos ao longo do ano (Oliveira; Gibbs, 2000) e fornece os recursos necessários para manutenção de ciclos reprodutivos múltiplos ao longo do ano.

Na região do Triângulo Mineiro, *X. frontalis* e *X. grisescens* são essenciais para a produção de frutos do maracujá-amarelo. Ninhos dessas espécies são mantidos em duas áreas de criação pertencentes a Universidade Federal de Uberlândia, nas quais são disponibilizados ninhos-armadilha de bambu para a atração de fêmeas nidificantes. Experimentos de introdução de ninhos ativos em

áreas de cultivo de maracujá-amarelo tem proporcionado o incremento na quantidade e qualidade dos frutos produzidos (Junqueira; Augusto, 2017).

Considerando o papel das abelhas na polinização do maracujá-amarelo e a influência do uso da terra nas populações desses insetos, o objetivo desse trabalho foi avaliar os efeitos dos diferentes grupos funcionais de visitantes florais na produção e qualidade dos frutos do maracujá-amarelo, em diferentes contextos da paisagem. Além disso, considerando apenas os polinizadores, avaliamos a dinâmica populacional e os comportamentos de nidificação em áreas de criação já estabelecidas. Também introduzimos e monitoramos ninhos ativos em áreas naturais e agrícolas com o objetivo de analisar o desenvolvimento desses ninhos e contribuir para o manejo dessas abelhas visando a conservação e o uso em polinização assistida.

No primeiro capítulo, avaliamos o efeito do uso da terra no entorno dos cultivos na abundância de visitantes florais e produção de frutos do maracujá-amarelo (*Passiflora edulis* f. *flavicarpa*) no Cerrado Mineiro. Foram avaliados os efeitos do uso do solo na abundância dos diferentes grupos funcionais de abelhas que visitam o maracujá-amarelo e nos serviços polinização prestados pelas abelhas *X. frontalis* e *X. grisescens*, os polinizadores efetivos do maracujá-amarelo. Adicionalmente, a diferença na produção de frutos por polinização manual e natural foi avaliada para análise do déficit de polinização observado nos cultivos.

No segundo capítulo, foi avaliada a dinâmica populacional de *X. frontalis* e *X. grisescens* em áreas de criação, com o objetivo de identificar possíveis diferenças comportamentais e na fenologia da nidificação entre essas espécies. Esse capítulo visa elucidar uma questão que já vem sendo discutida (ver Araújo *et al.* 2021) sobre o quanto essas espécies se sobrepõem em relação às diferentes dimensões do nicho ecológico. Assim, além de elucidar questões ecológicas, esse capítulo visa contribuir com projetos futuros de polinização assistida.

No terceiro e último capítulo, o objetivo deste foi avançar na compreensão do processo de colonização de ninhos-armadilha (NA) por fêmeas nidificantes de *X. frontalis*, em pequenos abrigos de abelhas instalados tanto em remanescentes naturais de Cerrado, quanto em cultivos de maracujazeiro.

Foram comparadas as taxas de colonização dos NA nessas áreas considerando duas situações: (i) em abrigos de abelhas contendo apenas ninhos-armadilha e (ii) após a introdução de um ninho ativo. Nossa expectativa era que a taxa de colonização seria maior em áreas naturais, menos impactadas por ações antrópicas e na situação (ii), devido aos comportamentos filopátricos realizados pelas fêmeas emergentes. Adicionalmente, observações sobre o comportamentos de nidificação foram realizadas.

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## CAPÍTULO 1

**Does the land use influence floral visitors' abundance and fruit set of passion fruit (*Passiflora edulis* f. *flavicarpa*) orchards in Brazilian Savanna?**

## Resumo

Nos agroecossistemas tropicais, 70% das culturas produzem em maior quantidade ou com melhor qualidade quando adequadamente polinizadas. O déficit na polinização pode diminuir o rendimento devido à falta de compatibilidade entre a morfologia floral ou o tamanho do polinizador, bem como fatores ambientais como solo, temperatura e outros fatores relacionados à paisagem que circunda a área de cultivo, como a fragmentação de áreas naturais. O maracujá é uma importante cultura comercial com alto valor comercial que apresenta um sistema reprodutivo complexo com dependência essencial de polinização cruzada mediada por abelhas de grande porte. Realizamos um estudo em onze pomares comerciais de maracujá entre 2020 e 2022 para avaliar como o uso da terra afeta a abundância de visitantes pertencentes a diferentes grupos funcionais de abelhas e, conseqüentemente, na produção de frutos do maracujá-amarelo. Também investigamos a diferença na produção de frutos por polinização manual e natural para análise do déficit de polinização observado nos cultivos.

Houve impacto positivo significativo da taxa de visitação de *Xylocopa frontalis* (polinizador efetivo) na produção natural de frutos de maracujá. Em relação aos pilhadores, curiosamente *Trigona* spp. mostraram um efeito significativo e positivo na polinização natural de frutos enquanto a taxa de visitação de *A. mellifera* teve um efeito negativo. Quanto ao uso da terra, áreas de uso moderado do solo, como pastagens, estão positivamente relacionadas a um maior produção de frutos, enquanto áreas de uso intenso do solo, como cultivos de soja, influenciam negativamente essa produtividade. Além disso, a maior presença de cobertura vegetal foi positivamente correlacionada com *Xylocopa frontalis* e negativamente correlacionada com *Apis mellifera*, enquanto o uso intenso do solo apresentou o comportamento oposto, sendo positivo para *Apis mellifera* e negativo para *Xylocopa frontalis*. Considerando o exposto, planejar cultivos e estratégias de manejo longe de áreas de uso intensivo do solo e próximos a pastagens e áreas naturais é uma prática amigável às abelhas que pode favorecer o rendimento do maracujá e ajudar a manter a presença de abelhas *Xylocopa* e deslocar as abelhas *Apis mellifera* dos pomares de maracujá.

**Palavras-chave:** polinização, maracujá, uso da terra, abelha carpinteira, mamangava de toco, formação de frutos, qualidade dos frutos.

## Abstract

In tropical agroecosystems, 70% of crops produce more or with better quality when adequately pollinated. Pollination deficits can decrease yield due to lack of compatibility between floral morphology or pollinator size, as well as environmental factors such as soil, temperature, and other landscape-related factors surrounding the cultivation area, such as natural area fragmentation. Passion fruit is an important commercial crop with high commercial value that presents a complex reproductive system with essential dependence on cross-pollination mediated by large bees. We conducted a study in eleven commercial passion fruit orchards between 2020 and 2022 to evaluate how land use affects the abundance of visitors belonging to different functional groups of bees and, consequently, in the production of yellow passion fruit. We also investigated the difference in fruit production by manual and natural pollination to analyze the observed pollination deficit in the crops.

There was a significant positive impact of the visitation rate of *Xylocopa frontalis* (effective pollinator) on the natural production of passion fruit. Regarding robbers, interestingly *Trigona* spp. showed a significant and positive effect on natural fruit pollination while the visitation rate of *A. mellifera* had a negative effect. Regarding land use, moderate soil use areas, such as pastures, are positively related to higher fruit production, while intensive soil use areas, such as soybean crops, negatively influence this productivity. In addition, the greater presence of vegetation cover was positively correlated with *Xylocopa frontalis* and negatively correlated with *Apis mellifera*, while intensive soil use presented the opposite behavior, being positive for *Apis mellifera* and negative for *Xylocopa frontalis*. Considering the above, planning crops and management strategies away from intensive soil use areas and close to pastures and natural areas is a bee-friendly practice that can favor passion fruit yield and help maintain the presence of *Xylocopa* bees and displace *Apis mellifera* bees from passion fruit orchards.

**Keywords:** pollination, passion fruit, land use, carpenter bees, fruit set, fruit quality.

## 1. Introduction

Agroecosystems depend strongly on a set of ecosystem functions provided by nature, in the unmanaged ecosystems. These services include genetic biodiversity, nutrient cycling, and the provision of water. However, depending on management practices, agriculture can also be the source of numerous disservices, such as the loss of wildlife habitat and pesticide poisoning of humans and non-target species (Power 2010). Understanding ecosystem disservices is important for the proper management of environments and for informed decision-making about land and natural resource use (Ferraz *et al.*, 2019).

Pollination is an essential ecosystem function that plays a crucial role in plant reproduction, agricultural production, and consequently the maintenance of terrestrial ecosystems (Ratamäki *et al.*, 2015). It indirectly affects several ecosystem services, including food production and the guarantee of reliable and diversified supply of fruits, seeds, honey, among others, and the promotion of cultural values related to traditional knowledge (Costanza *et al.* 1997). It involves the transfer of pollen grains between the male and female flowers organs, that can be carried out by animals, wind, or water. The majority of plants, both cultivated and native, rely on animal pollinators for their reproduction (Klein *et al.*, 2007, Ollerton *et al.* 2011; Roubik 2018). In tropical communities, 94% of plants are pollinated by animals (Ollerton *et al.* 2011), mainly insects such as bees, flies, butterflies, moths, wasps, beetles, and trips. When considering only cultivated plants that are pollinated by animals, 70% of the total of 1,330 crops in tropical regions produce a greater quantity and/or better quality of fruits and seeds when adequately pollinated (Roubik 2018).

The deficit in crop pollination, that refers to the inadequate or insufficient transfer of pollen, decreases yield. This can result from factors such as insufficient or inefficient pollen transfer due to limited pollinator abundance and diversity, efficiency, or flowering asynchrony (Vaissière *et al.* 2011) and can be caused by a lack of compatibility between floral morphology or pollinator size, as well as environmental factors like soil, temperature, and other landscape-related factors surrounding the cultivation area, such as the fragmentation of natural areas (Wolowski, 2019).

In crops such as acerola (*Malpighia emarginata*), passion fruit (*Passiflora edulis*), and Brazil nut (*Bertholletia excelsa*), medium to large-bodied specialized bees are required to collect resources offered by these species for fruit and seed formation (Freitas et al. 1999; Gaglianone et al. 2010; Cavalcante et al. 2012; Vilhena et al. 2012; Oliveira et al. 2014; Junqueira and Augusto 2017).

Passion fruit is a highly valued and edible fruit with a high commercial value. Between 2017 and 2021, the production of passion fruit in Brazil experienced significant growth, with an increase of over 50% in the national production value, from less than 171 million USD to 305 million USD annually (IBGE agricultural census 2021). The largest national producers are the states of Bahia, Ceará, Santa Catarina, and Minas Gerais, respectively. Bahia is the largest producer (in tons produced) due to its disproportionate planted area (16,816 hectares), but not due to the state's yield (12 tons per hectare), which is lower than production observed for other states such as Ceará (24 tons per hectare), Santa Catarina (25 tons per hectare), and Minas Gerais (17 tons per hectare). The city of Araguari, in the Triângulo Mineiro region, presented an average production of 21 tons per hectare (IBGE agricultural census 2021), well above the national production of 15 tons per hectare. Furthermore, in Brazil passion fruit crops is also of great social importance, being usually cultivated by small farmers who use the fruits as a complementary source of income, selling them fresh or to fruit juice processing industries (Yamamoto *et al.*, 2010, Oliveira *et al.* 2014).

The flowers of this specie have a complex reproductive system. It has typical melittophilous characteristics (Yamamoto et al. 2012) with perfect, large, colorful, fragrant flowers that produce abundant nectar (Akamine and Girolami 1959) and an essential dependency on pollination (Wolowski et al. 2019). As a result, these flowers cannot produce fruit through spontaneous self-pollination or manual self-pollination. Therefore, cross-pollination is necessary, requiring the presence of suitable pollinators to transport pollen between flowers (Ruggiero 1973).

In a study conducted in the Brazilian savanna of Triângulo Mineiro, Yamamoto et al. (2012) recorded 27 bee species on passion fruit flowers in commercial orchards. The floral visitors were classified into three groups based on whether they touched the stigma of the flowers: pollinators, occasional pollinators, and robbers, with 17 pollinators, including 12 effective and 5 occasional. The most



effective pollinators were large solitary bees, such as *Xylocopa frontalis*, *X. grisescens*.

Bees of genera *Xylocopa* are the most efficient passion fruit pollinators (Corbet and Willmer 1980; Sazima and Sazima 1989; Camillo 2003, Pereira and Garófalo 2010, Yamamoto et al. 2012, Junqueira et al. 2012 and 2013, Junqueira and Augusto 2017), because of their large size and their behavior during flower's foraging (Camillo et al. 1986).

The large bees can overcome the membranous barrier present in nectar chambers, and can spend minutes collecting nectar, often rotating around the androgynophore, surrounding the flower beneath the anthers in a rotation behavior. As doing it, the larger bees, come in contact with the flower's anthers using their thorax. As they move around the nectar chamber, they deposit some of the pollen collected from their prior visits onto the stigma of the flower. When these bee visits coincide with the alignment of reproductive organs, the chance of effective pollination increases, even with few visits (Silva et al. 2014). On the other hand, small bees such as *Apis mellifera* and *Trigona spinipes*, categorized as robbers, cut through the nectar chamber membrane to access the nectar, damaging the flower, and are considered detrimental to yield (Oliveira et al. 2014).

*Xylocopa* bees are a type of above-ground nesting bees (Vaughan et al. 2014). Most *Xylocopa* species excavate nests above ground in dead wood, although some species also frequently nest in pre-existing cavities such as the culms of plants (Buchmann et al. 2019). These bees are solitary or facultatively social and have been studied in trap-nests for over 30 years in Brazil (Camillo and Garófalo 1982; Camillo, Garófalo and Muccillo 1986; Camillo and Garófalo 1989; Viana et al. 2001; Camillo 2003, Bernardino and Gaglianone, 2013, Marchi and Melo 2010, Chaves-Alves et al. 2011, Pedroso et al. 2021, Souza et al. 2021).

The presence of *Xylocopa frontalis* and *Xylocopa grisescens* in crop areas resulted in an increase in both production and quality of passion fruits, as well as a reduction in the presence of *Apis mellifera* (Junqueira et al. 2013; Junqueira and Augusto 2017). However, the rational breeding of these species remains a major challenge and is not yet commercially effective. Therefore, further research

is needed to develop more cost-effective and sustainable strategies for the management of *Xylocopa* bees in agroecosystems to increase the productivity of this important crop for agriculture in Brazil. While hand-cross pollination has been used to minimize the pollinator deficit of passion fruit in farms, this practice is expensive and raises production costs up to 12% (Junqueira et al. 2013).

Usual agricultural practices heavily rely on the intensive use of pesticides and extensive monoculture, which threaten pollination services (Park et al. 2015). These practices also affect the stability and resilience of agricultural systems, leading to a lower capacity for effective management strategies (Nelson et al. 2010). Moreover, land cover changes resulting from agricultural practices promote the loss of natural habitats and threaten pollinators, impacting the quality and quantity of food resources and the availability of nesting sites in the environment (Barbosa 2020).

In this study, we examined how the abundance of floral visitors and their functional groups affect the fruit set of passion fruit (*Passiflora edulis* f. *flavicarpa*) orchards, as well as the impact of the spatial pattern of land use intensity on both parameters in Brazilian savanna. We hypothesize that the intense soil use in the vicinity of crops negatively affects the native bees (pollinators or robbers), that depends on natural habitats to nidification, and had a neutral effect to the exotic bee *Apis mellifera* (robbers). We also investigate how the treatments of pollination, and the deficit categories of those orchards influence the quality of fruit production.

## **2. Material and Methods**

### *2.1 Floral biology of passion fruit*

The passion fruit flower is monoclinal, self-incompatible, and protandrous, with the male floral parts moving at the beginning of anthesis and the female parts subsequently (Ruggiero et al. 1996, Camillo 2003, Oliveira et al., 2014). This complex self-incompatibility system results in a strict dependence on pollinator services (Rêgo et al. 2000). The maximum lifespan of each flower is six hours,

and the corolla closes at dusk. The stigmatic receptivity and pollen viability periods overlap during anthesis (Melo *et al.* 2014).

Floral visitors are drawn to the areas by the abundant nectar availability in nectar chamber. Nectar production begins to rise after anthesis, reaching its peak two hours later. Still, from 3:30 PM onwards, there is a gradual decrease in nectar availability, continuing until the end of the day. The studied variety, FB-300, reaches its peak nectar production of 20 $\mu$ L at 2:30 PM, and this resource availability drops to 4.8 $\mu$ L by 5:30 PM (Cobra *et al.*, 2017). The volume of nectar removed from bagged flowers in pre-anthesis, and the total volume produced in flowers after successive removals did not differ in passion fruit flowers. This demonstrates that the removal of nectar does not stimulate its secretion and suggest that the reduction in visits in passion fruit in the end of afternoon may be related to the lower availability of nectar (Melo *et. al.* 2014)

Passion fruit flowers have a high production of pollen grains, with more than 300 seeds on average, and the requirement of pollen to properly fertilize each ovule is quite high (Viana *et al.*, 2014).

## 2.2 Study areas

We conducted this study in eleven commercial orchards of yellow passion fruit of the cultivar 'FB300 - Araguary', a rustic cultivar developed for Savanna regions and produced by 'Viveiro Flora Brasil'. This commercial hybrid is selected for industrial juice production with non-uniform sizes, shapes, and colors (yellow or purple).

All areas were in the surrounding area of Araguari city, which is situated in the Triângulo Mineiro region of the State of Minas Gerais, Brazil (Table 1; Figure 1). In 2021, Araguari was the largest producer of passion fruit in the state, ranking 48th in the country, producing 2,300 tons on 110 hectares, with an average yield of 21 tons per hectare. In the region, soybean is the primary agricultural production, typically cultivated in extensive monocultures. Additionally, coffee, tomato, and cattle farming are major agricultural activities (IBGE 2021).

The municipality is located in the Brazilian savanna, in a region with spots of Atlantic Forest (Ribeiro and Walter, 1998; Morellato and Haddad, 2000; Vale *et al.* 2009). Part of our study areas are included in the Atlantic Forest biome

legislation under Law No. 11.428/2006 (IDESISEMA), encompassing Semideciduous Seasonal Forests and areas under ecological tension of Atlantic Forest. The climate of the region is tropical, with two well-defined seasons: the rainy summer (October to March) and the dry winter (April to September) (Rosa *et al.*, 1991).

All study areas were small orchards (<6 ha) where the passion fruit was not the only crop or product, and the areas were at least 6km apart from each other. Field observations and pollination experiment were carried out only in rainless days during the passion fruit flowering periods from 2020 to 2022. The average temperature in the sampling days ranged from 26,8°C to 38,4°C and the humidity from 34% to 62%.

To characterize the bee community visiting yellow passion fruit areas and their flower behaviors, we conducted field observations. All areas were sampled once, and it was possible to resample five of the areas (MCJ2, MCJ4, MCJ5, and MCJ6) the following year, totaling 15 visitors' observations. For the pollination experiment (fruit set and quality), we analyzed data from 10 areas (MCJ2, MCJ3, MCJ4, MCJ5, MCJ6, MCJ7, MCJ8, MCJ9, MCJ10, MCJ11), without replications, and investigate the relationship between these floral visitor bees and the fruit production of yellow passion fruit orchards.

**Table 1.** Passion fruit orchard's location and size, in hectare.

Area	Municipality	Longitude	Latitude	Area (ha)
MCJ1	Araguari	-18.512.336	-48.021.075	2,56
MCJ2	Indianópolis	-18.937.225	-47.898.115	1,59
MCJ3	Indianópolis	-18.875.548	-47.979.789	0,71
MCJ4	Araguari	-18.569.470	-48.018.382	0,80
MCJ5	Araguari	-18.806.247	-47.982.315	6,00
MCJ6	Araguari	-18.584.159	-48.407.585	2,00
MCJ7	Araguari	-18.550.547	-48.170.277	1,44
MCJ8	Araguari	-18.521.225	-48.242.806	1,89
MCJ9	Araguari	-1.863.737	-4.805.606	0,30
MCJ10	Araguari	-18.641.500	-48.259.000	1,18
MCJ11	Uberlândia	-189.463	-4.805.188	1,68

### 2.3 *Observations about behavior of bees' visitors*

During field observations in the blooming periods, we recorded the abundance and diversity of bee visitors for 10 minutes every hour, from 1:00 PM to 6:00 PM (5hrs), in two rows of growing lines measuring 50 meters. We visually identified the bees to the highest possible taxonomic level.

We estimate the frequency of occurrence (OF) of visitors as the percentage of number of occurrences of each species by the total number of samples (Buschini 2006) and considered bees with  $OF \geq 50\%$  as highly frequent (HF), between 25% and 50% as frequent (F) and below 25% as less frequent (LF).

To investigate the behavior of bees during flower visits, we examined the collected resources (pollen or nectar) and the approach these bees employed to access these resources (legitimate or illegitimate pollination visits). We also documented whether direct contact occurred between the thorax of the bee and the anther of the passion fruit flower in the large bees. For each species under analysis, we calculated the percentage of contact with the anthers of passion fruit flowers to determine the effectiveness of pollen deposition (EPD).

Based on the analysis of flower visit behaviors, we categorized the bees into five distinct groups: effective pollinators, occasional pollinators, neutral visitors, robbers, and nectar robbers. To be classified as effective pollinators, the bees had to exhibit highly frequent (HF) visitation rates and demonstrate high effectiveness of pollen deposition (EPD), with at least 75% of effectiveness in the observed cases.

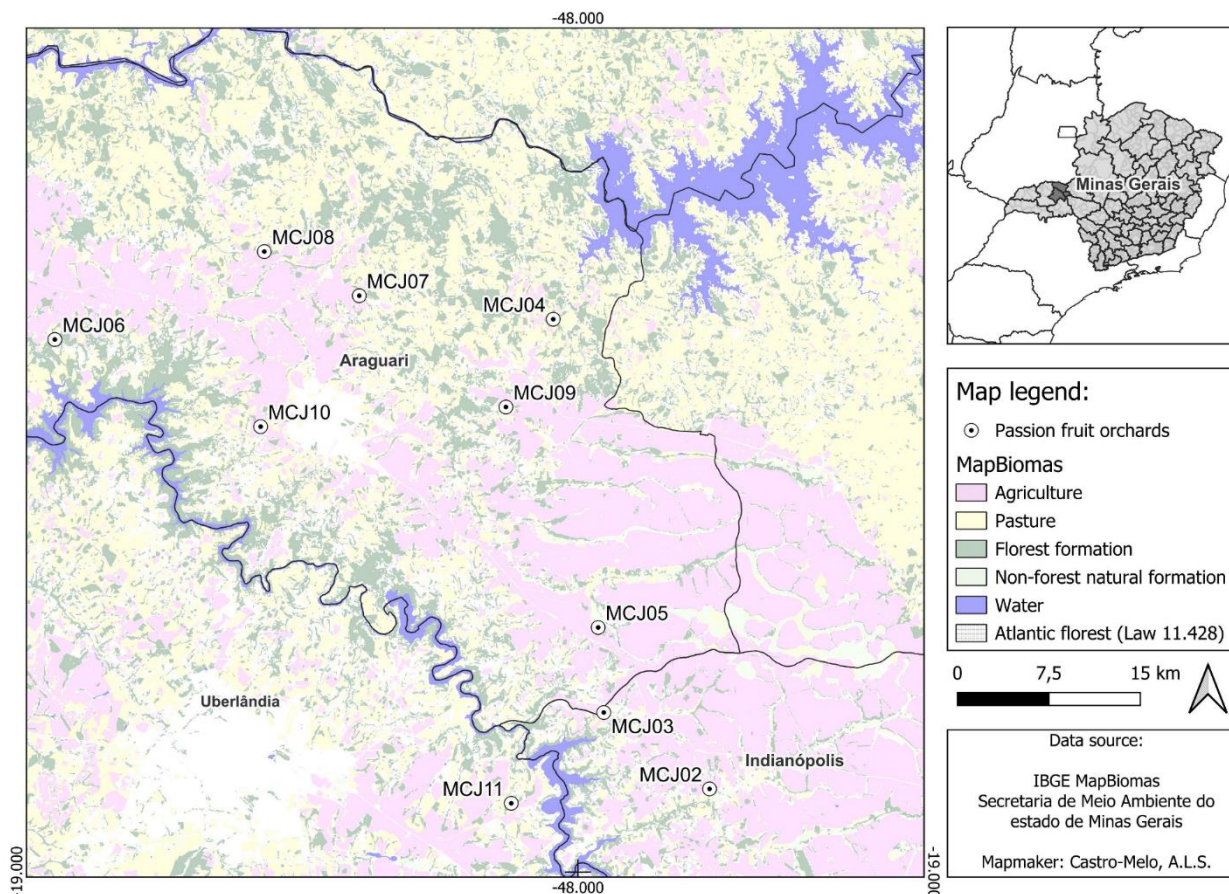


Figure 1. Geographic location of passion fruit crops with the coverage distribution of land use between Araguari, Uberlândia and Indianópolis, in Triângulo Mineiro region of Minas Gerais state, Brazil.

#### 2.4 Fruit set and quality analysis

We evaluated the abundance of floral visitors in the passion fruit areas using the transect census method (Dafni *et al.*, 2005; Winfree *et al.*, 2008). In each study area, we established two transects of 50 meters long transects along the crop lines. These transects were systematically surveyed for 10 minutes each, at hourly intervals, spanning from 1:00 PM to 6:00 PM with a sampling effort of 5 hours per study area. The assessment of passion fruit set was conducted through pollination experiments within each study area.

The hand-cross pollination treatment is a way to observe the result of managing the cultivated area and the agricultural factors and corrections applied to the soil. This method involves manually pollinating all the flowers in the orchard to achieve the optimal potential of that orchard. On the other hand, natural

pollination demonstrates the differences between the activity of pollinators in that area. Hand-cross pollination was performed by applying pollen from distinct individuals to 50 flowers, starting within the first 2 hours after anthesis and natural pollination was measured by marking 100 open flowers and leaving them open for bee visitation within the analyzed crop lines (Figure 2). The success of pollination was observed around five days after the visit, using fruit formation as a criterion (Melo *et al.* 2014). These pollination experiments were made during the peak flowering period with, at least, 160 flowers in the two lines, which displayed enough number of open flowers to attract bee species.

The fruit quality was evaluated in nine of the ten areas used to pollination experiments. We analyzed 139 fruits, 59 of natural pollination and 80 of hand-cross pollination from all areas. The MCJ10 was abruptly discontinued before reaching the end of the fruit development period. We measure the fruit weight (g), peel weight (g), pulp content (seeds with aril), and the respective percentages of peel and pulp. Additionally, we assessed the soluble solids (SS) by the sugar content of the fruit juice using a manual refractometer, with results expressed in Brix°.

### *2.5 Deficit categories and land use in the vicinity of passion fruit*

All study areas were categorized according to its deficit of pollination based on the observed natural pollination percentages from pollination experiments. The classification was based on the most recent assessments of passion fruit production in the region (Yamamoto *et al.*, 2012; Junqueira *et al.*, 2016). In these studies, the average natural pollination percentage for passion fruit stands at 16.2% (Yamamoto in 2012 = 23.3%; Junqueira in 2016 = 10.6%). Thus, passion fruit orchards showing natural pollination percentages exceeding 16% were classified as areas exhibiting low pollination deficits (LPD), while those with percentages below 16% were categorized as areas with high pollination deficits (HPD).

To assess the land cover structure in the surrounding areas of passion fruit orchards, we utilized the MapBiomas project, which provides detailed land use categories for the entire Brazilian territory. We determined the class values for buffers of one and four kilometers. The classes include forest formation, savanna formation, non-forest formation, pasture, mosaic of agriculture and pasture, and agriculture subdivided into temporary crops, perennial crops and forest plantation. Image processing was conducted using QGIS® 3.32.1 (Figure 3 to Figure 6).



**Figure 2.** Pollination treatments (a) hand-cross pollination (b) natural pollination. (c) Transect in passion fruit orchard.

To describe and analyze the land use coverages and their relation with fruit set and the abundance of bees' visitors in the orchards and the fruit set, we distributed them into three groups: vegetation cover, consisting of forest formation, savanna, and non-forest natural formation; moderate soil use, including pasture and mosaic of pasture and agriculture; and intense soil use,



encompassing perennial crops, forest plantation and temporary crops. We also compared the natural pollination to cross-hand pollination percentages to analyze the differences between the low pollination deficits (LPD) and high pollination deficits (HPD).

## 2.5. Data analysis

### *Bees' abundance and natural pollination of passion fruit*

The bee abundance of the ten areas of passion fruit pollination experiment were pooled for statistical analysis. We used Generalized Linear Models (GLM), with R-package *glmmTMB* version 1.0.2.1 (Brooks et al., 2017), to test if natural pollination were affected by the Shannon-Wiener Diversity Index ( $H'$ ), the visitation rate of all pollinators (effective and occasional pollinators), all robbers (robbers and nectar robbers) and the high frequency (HF) visitors (according to frequency of occurrence), individually. As the data was the proportion of natural fruit set, we applied a beta family of *glmmTMB*. Using the R-package *DHARMA* version 0.4.1 (Hartig, 2020) we confirmed the model fit after residual check and overdispersion test.

Mixed models were used to test the effect of flower visitors' rates on natural pollination of passion fruit. We used Akaike Information Criteria (AIC) for adjust the model to its most parsimonious form using R-package *MASS* version 7.3 (Venables & Ripley, 2002). We applied model selection for the null model, the full model (with all variables describe above) and the adjusted model using the corrected Akaike's Information Criterion (AICc) (Burnham and Anderson, 2010). The model that minimizes AIC ( $AIC \leq 2$ ) provided the most reliable fit to the data. Model's significance was inferred using type II likelihood ratio tests with R-package *car* version 3.0.10 (Fox and Weisberg, 2019).

### *Effect of fruit set quality by natural pollination and cross-hand pollination*

Before assessing the factors influencing fruit set quality, we examined multicollinearity among potential continuous explanatory variables: fruit weight, pulp weight, peel weight, percentage of pulp content, percentage of peel, and

soluble solids. We utilized the R-package *PerformanceAnalytics* version 2.0.4 (Peterson *et al.*, 2020) to calculate the variance inflation factors (VIF) for these variables. A VIF above 3.0 indicated multicollinearity (sensu Zuur *et al.*, 2009; 2010). For our analysis, we used Linear Models (LM) to investigate whether treatment (natural pollination and hand-cross pollination), deficit classification (HPD and LPD), or their interaction were correlated with the variation in percentage of pulp content and soluble solids, separately.

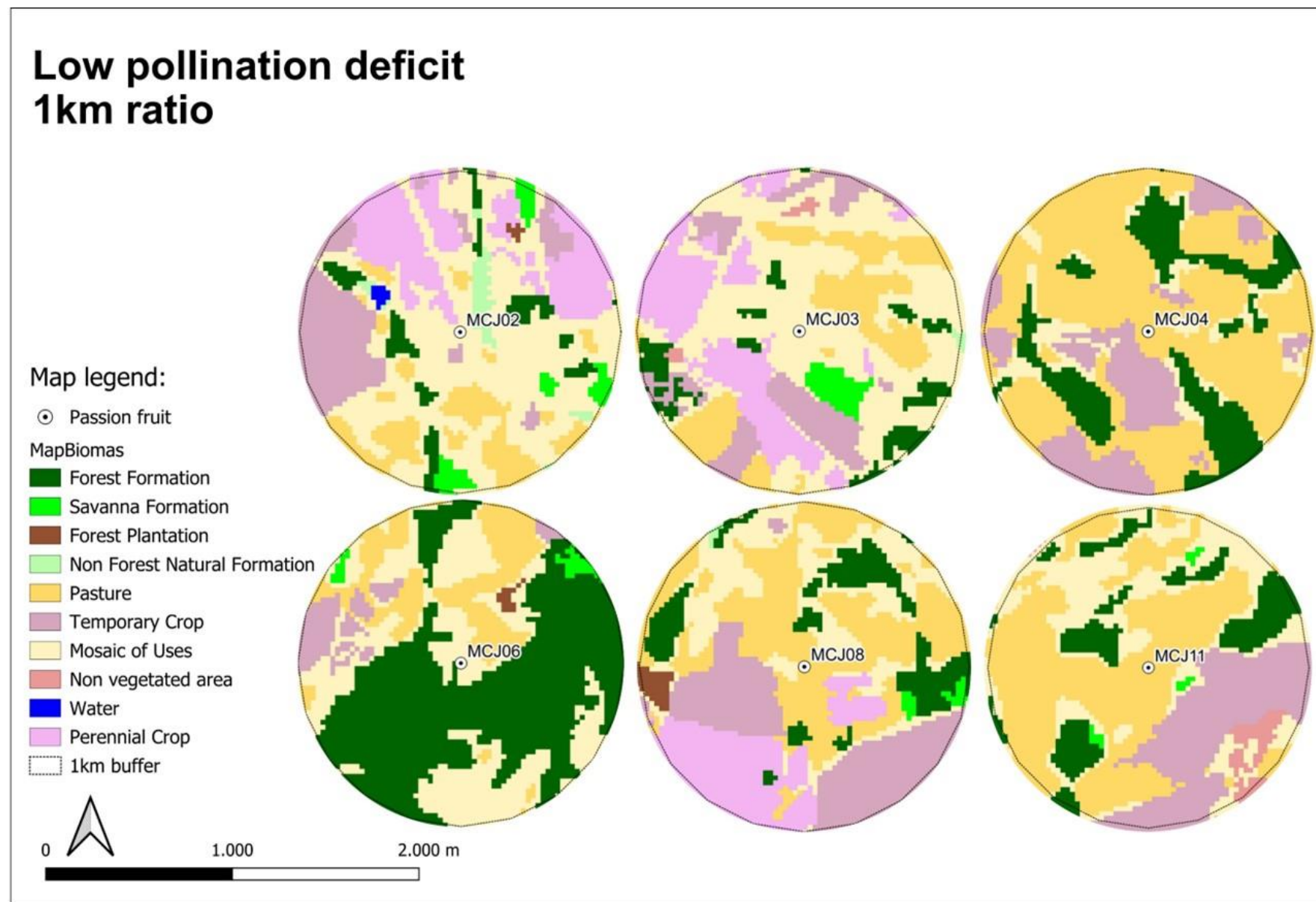
#### *Influence of land use in natural pollination of passion fruit*

We used Generalized Linear Models (GLM) with the *glmmTMB* package version 1.0.2.1 (Brooks *et al.*, 2017) to investigate the effect of land use coverage in the surrounding areas of passion fruit on natural pollination within one and four-kilometers buffer ratios. Savanna, non-forest formation, and forest plantation had average coverage indices below 3% in all areas and were not used in the models. Therefore, only forest formation represented the vegetation cover, while moderate land use comprised the pasture and mosaic of uses classes and intense land use included the perennial and temporary crops.

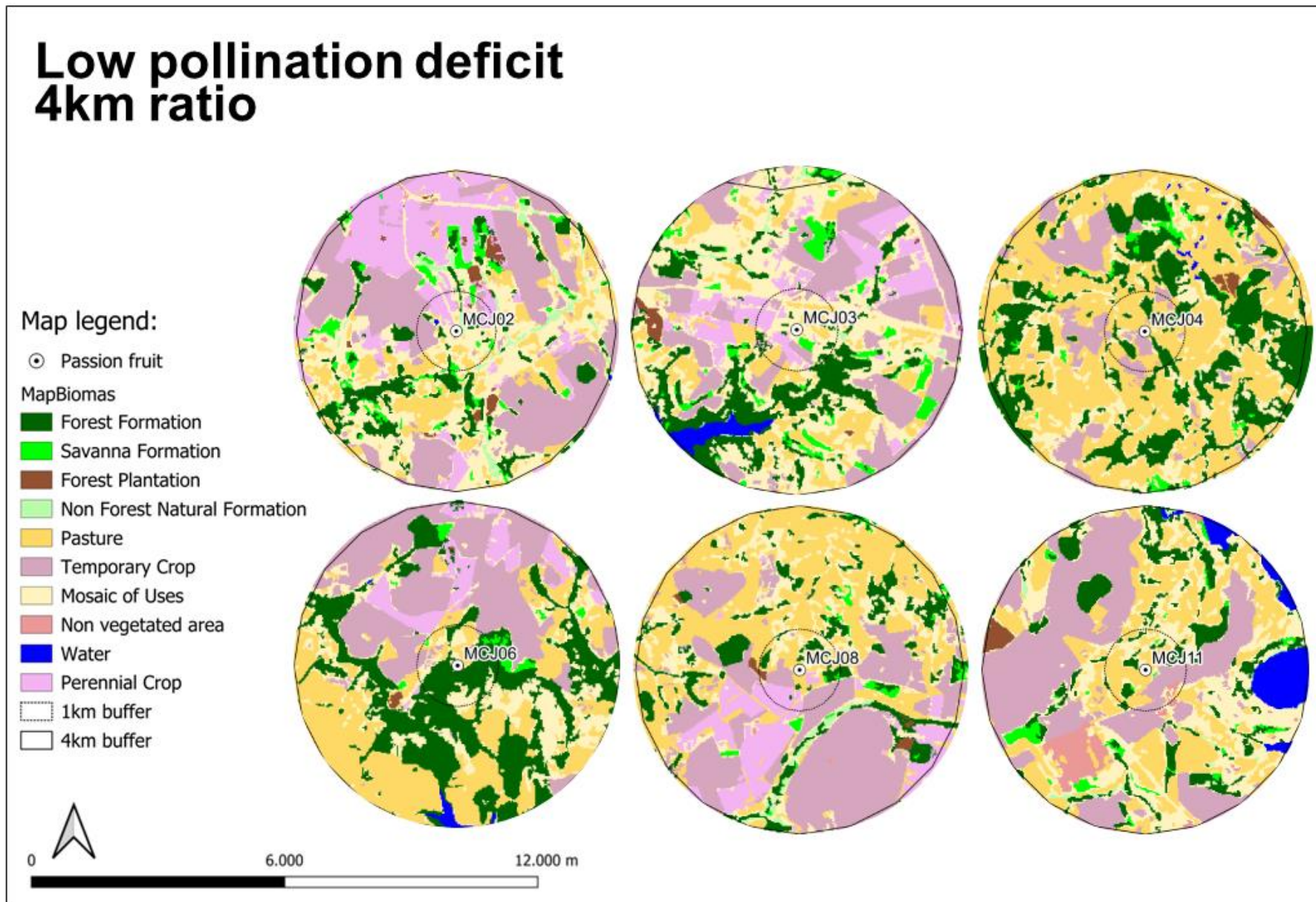
To analyze our model, we used a beta family distribution in *glmmTMB* because it is suitable for modeling proportions. We used *DHARMA* R-package to visualize model fit after residual check and overdispersion test.

We used Linear Models (LM) to analyze the relationship between the abundance of high-frequency bees in passion fruit orchards and the land use in the vicinity of the orchards. Our aim was to investigate whether the coverage of land use (vegetation cover, moderate soil use, and intense soil use) had a significant correlation with the abundance of these bees and the direction of this interaction. A positive correlation coefficient would indicate a positive relationship, meaning that as land use coverage increases, bee abundance also increases, and the reverse is also true for a negative correlation coefficient.

All statistical analyses were carried out using R version 4.1.0 (R Development Core Team, 2021).

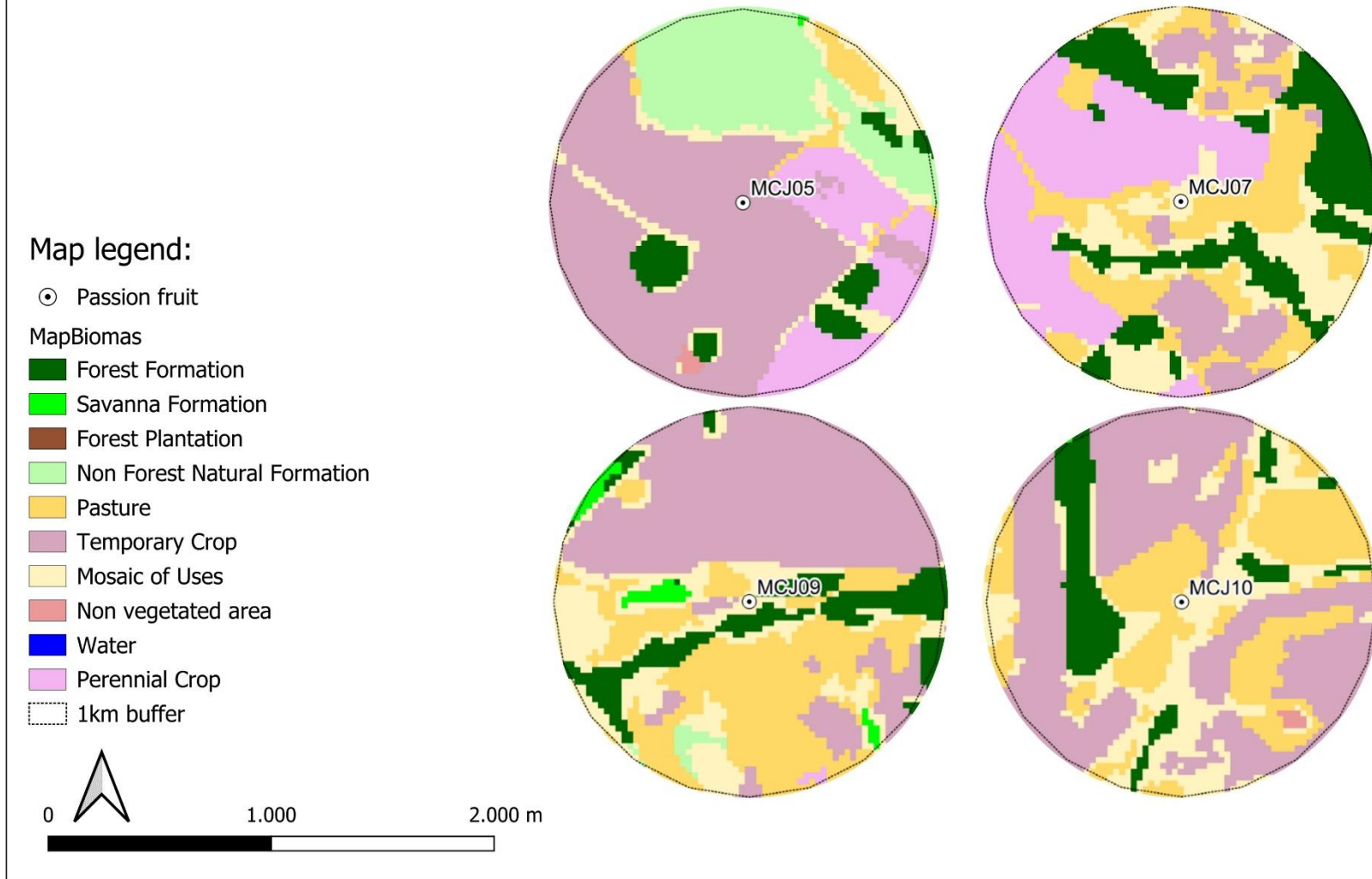


**Figure 3.** Land use coverage of natural areas (forest, savanna and non-forest natural formation), moderate soil use (pasture and mosaic of agriculture and pasture) and intense soil use (perennial crops, forest formation and temporary crops) in LPD areas in 1km ratio in Brazilian savanna.



**Figure 4.** Land use coverage of natural areas (forest, savanna and non-forest natural formation), moderate soil use (pasture and mosaic of agriculture and pasture) and intense soil use (perennial crops, forest formation and temporary crops) in LPD areas in 4km ratio in Brazilian savanna.

## High pollination deficit 1km ratio

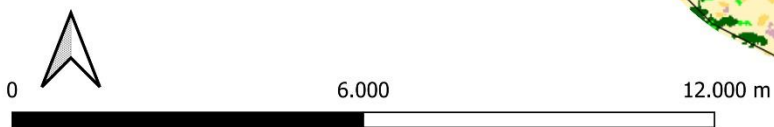
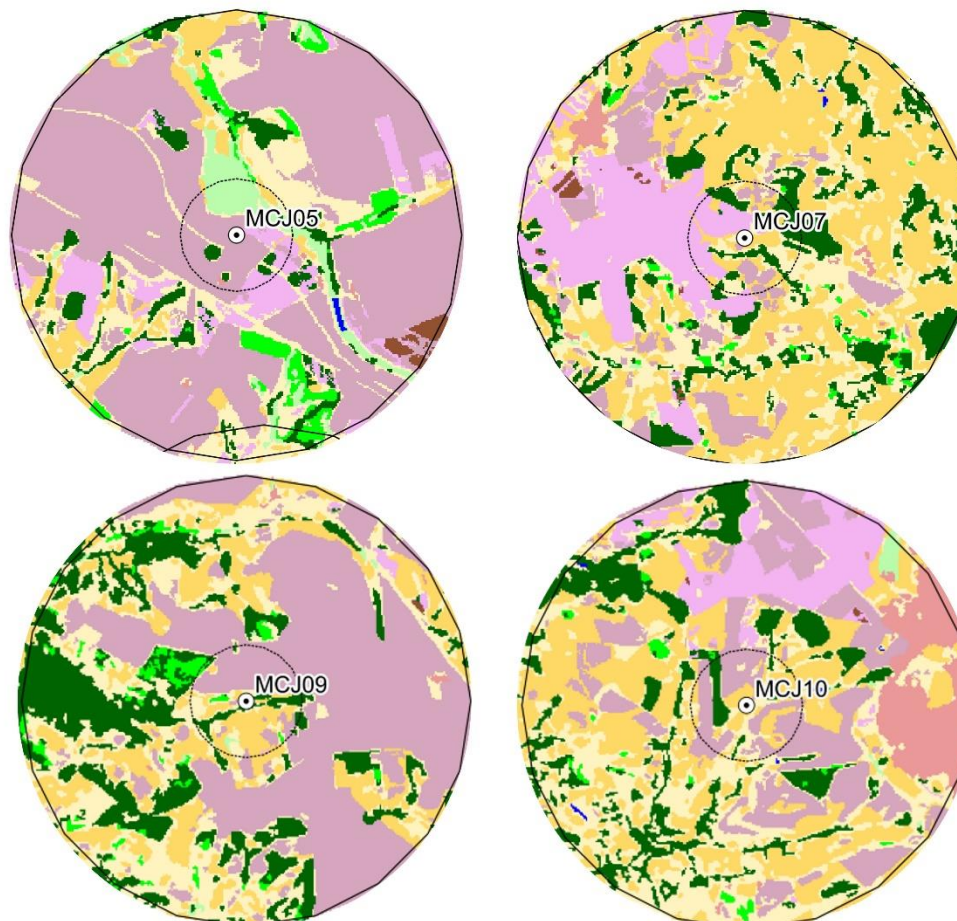


**Figure 5.** Land use coverage of natural areas (forest, savanna and non-forest natural formation), moderate soil use (pasture and mosaic of agriculture and pasture) and intense soil use (perennial crops, forest formation and temporary crops) in HPD areas in 1km ratio in Brazilian savanna.

## High pollination deficit 4km ratio

### Map legend:

- ⊙ Passion fruit
- MapBiomass
- Forest Formation
- Savanna Formation
- Forest Plantation
- Non Forest Natural Formation
- Pasture
- Temporary Crop
- Mosaic of Uses
- Non vegetated area
- Water
- Perennial Crop
- ⋯ 1km buffer
- 4km buffer



**Figure 6.** Land use coverage of natural areas (forest, savanna and non-forest natural formation), moderate soil use (pasture and mosaic of agriculture and pasture) and intense soil use (perennial crops, forest formation and temporary crops) in LPD areas in 1km ratio in Brazilian savanna.

### 3. Results

#### 3.1 Diversity of bees and functional groups

We recorded 2,950 bees belonging to seven tribes and identified at least 17 species, with native social bees identified up to the genus level. The most abundant floral visitor was the exotic social bee *Apis mellifera*, with 2,141 individuals representing 72.58% of the total abundance. This species was the only one present in all areas, with a highly frequent (HF) occurrence.

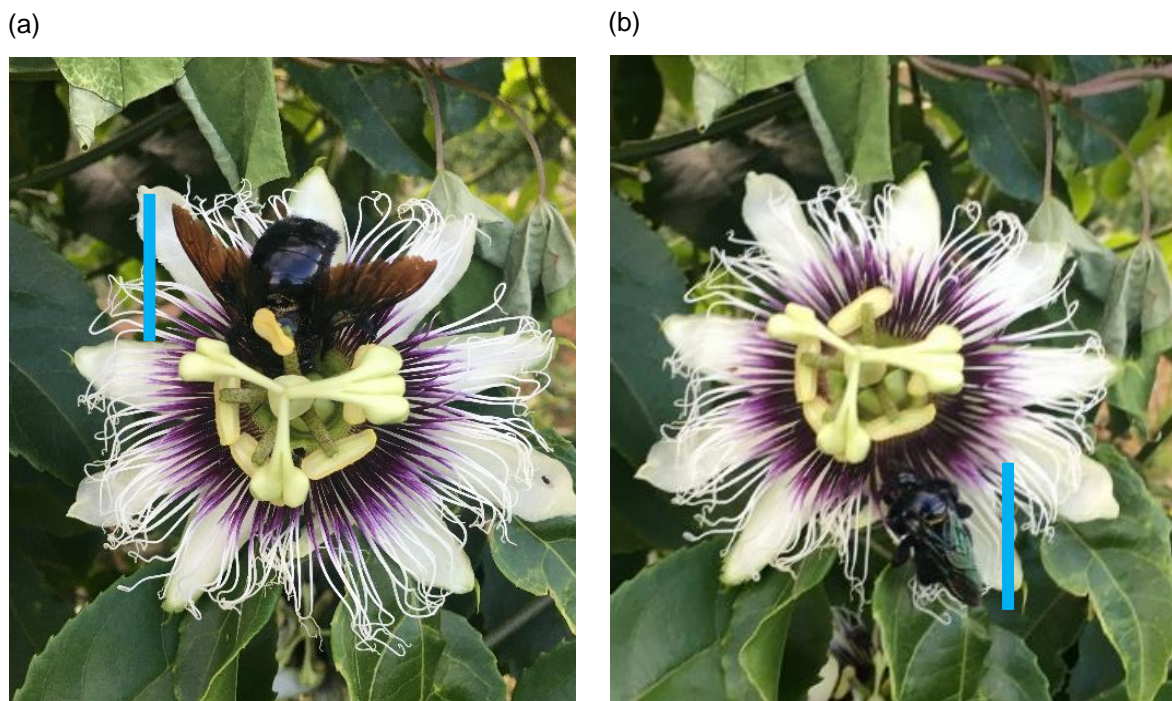
The *Trigona* genus, represented by *Trigona spinipes* and *Trigona hyalinata*, was the second most abundant, with 572 observations (19.39%) and also HF occurrence (14 of 15 areas). *X. frontalis* was the third most abundant species, with 62 individuals representing 2.10% of the total observed abundance and HF of occurrence (11 of 15 areas). We also observed 42 individuals of *Xylocopa suspecta*, representing 1.42% of the samples, with HF occurrence (10 de 15 áreas) (Table 2).

According to the resources collected, we observed that twelve of these species exclusively collected nectar, while the remaining five, all social bees, collected both nectar and pollen. The large bees (*Bombus*, *Centris scopipes*, *Epicharis flava*, *Xylocopa frontalis*, *Xylocopa grisescens* and *Xylocopa suspecta*) collected nectar legitimately, as well as *Eulaema nigrita*, *Euglossa* sp., and the bees of *Melipona* genus. *Apis mellifera* and species of genera *Paratrigona*, *Tetragona*, *Tetragonisca* and *Trigona* collected both resources in legitimate and illegitimate visits; *Oxaea austera* and *Oxaea flavescens* collected nectar but also in legitimate and illegitimate visits. All pollen-collecting bees were small-sized and necessarily engaged in illegitimate visits.

Examining the large bees, we found that *X. frontalis* (102), *X. grisescens* (4), *C. scopipes* (4), and the genus *Bombus* (3) presented effectiveness pollen deposition (EPD) in passion flowers, with over 75% of the observed visits. *Xylocopa suspecta* (66) and *E. flava* (9) contacted the anthers in 44% and 22% of the observed cases, respectively. The species *E. nigrita*, *O. austera*, and *O. flavescens* did not contact the anthers in any of the observations (Table 2).

Based on the data provided, we classified the pollinators into five distinct categories. The "*Effective pollinators*" contained exclusively *X. frontalis* (Figure 7a) in the studied region. This specie was categorized as effective pollinator because it presents a high effectiveness of pollen deposition (> 75%) and highly frequency of occurrence. In "*Occasional pollinators*" *X. suspecta* (Figure 7b) had a highly frequency of occurrence but not enough EPD. On the other hand, *X. grisescens* (Figure 8), *Bombus* spp. and *C. scopipes* presented high EPD but did not was present in sufficient frequency. *Epicharis flava* did not achieved any of criteria. The "*Neutral visitors*" was *E. nigrita*, *Euglossa* sp., and *Melipona* spp. All of them present LF of occurrence and collect resources without interferences in the pollination system.

In the "*Robbers*" category, the following social bee species were grouped: *A. mellifera* (Figure 9), *Trigona* genus (Figure 10), *Tetragona* genus, and *Tetragonisca* genus. Moreover, the "*Nectar Robbers*" category included bees that only collect nectar but also pierce the nectar chamber of the flowers. We classified *O. austera* and *O. flavescens* in this category (Table 2).



**Figure 7.** Size of *Xylocopa* bees observed in the study region. (a) *X. frontalis*; (b) *X. suspecta*. The blue line represents the same scale for comparisons.





**Figure 8.** Size of *Xylocopa grisescens*. The blue line represents the same scale for comparisons with Figure 7.

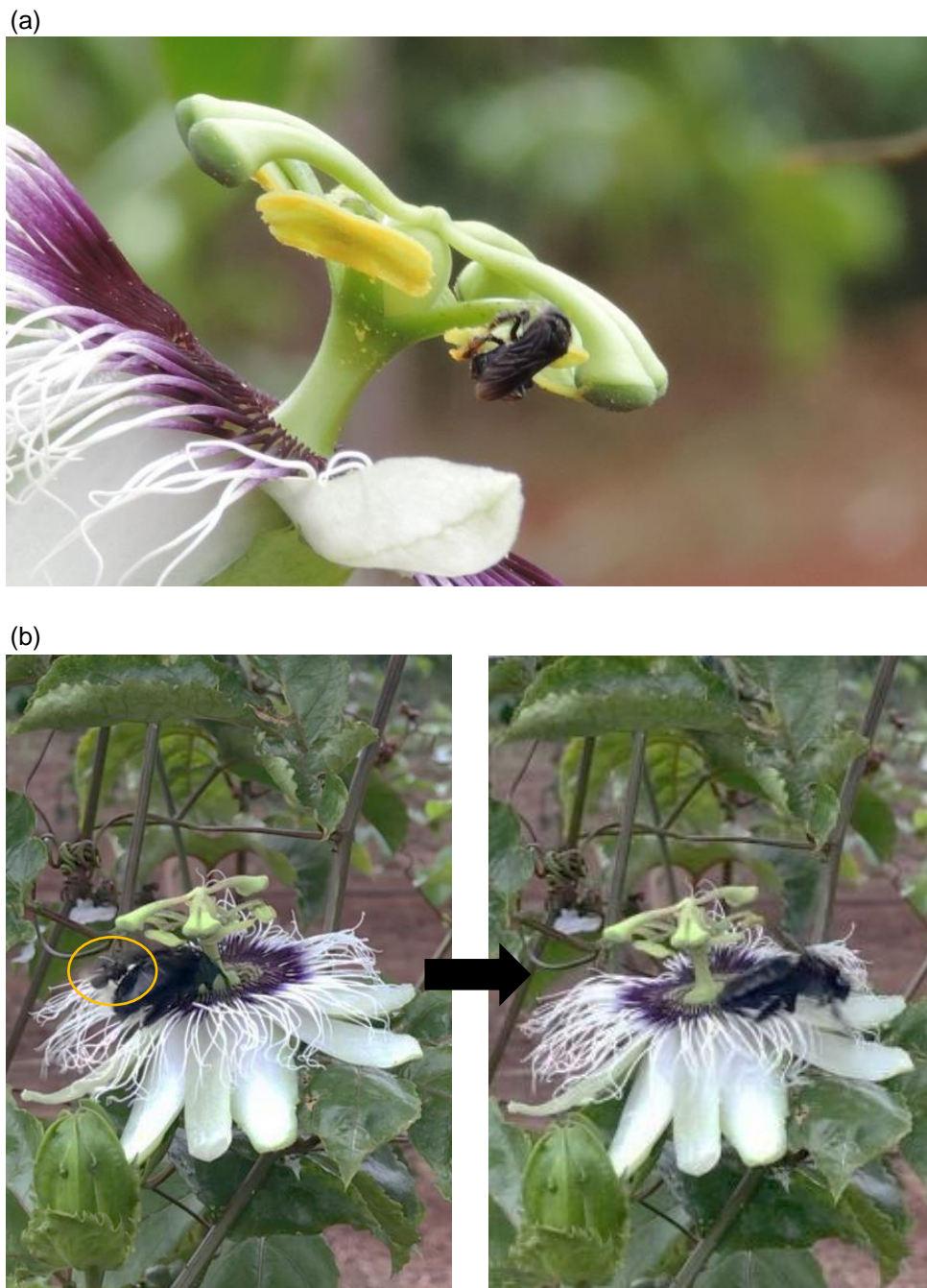
(a)



(b)



**Figure 9.** (a) Collection behavior of *Apis mellifera* in flower in anthesis in passion fruit flowers. (b) *Apis mellifera* collecting nectar from open passion fruit flowers whose anthers no longer have pollen.



**Figure 10.** (a) *Trigona* bee in a not frequent behavior of collecting pollen in passion fruit flowers. (b) Interaction record between *Xylocopa* and *Trigona* bees. The *Trigona*, in the yellow circle, is bit the wing of the *Xylocopa* and displaced it from the flower.

**Table 2.** Floral visitors on passion fruit (*P. edulis* f. *flavicarpa*) orchards in Triângulo Mineiro region, state of Minas Gerais, Brazil. Relative abundance (Ra); Frequency of Occurrence (FO); Effectiveness in Pollen Deposition (EPD).

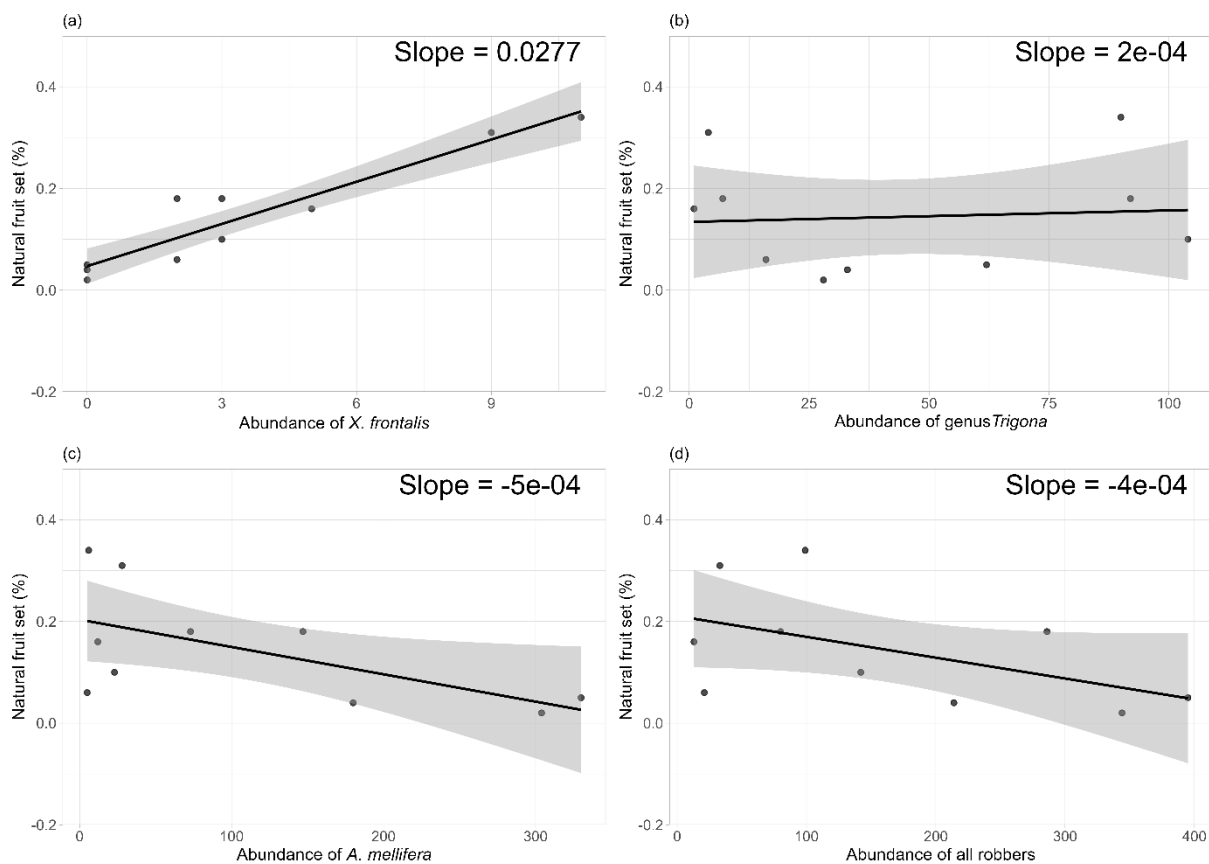
Bees visitor	Resource	Abundance (Ra)	OF	EPD	Classification
<b>Apini</b>					
<i>Apis mellifera</i>	Nectar/Pollen	2141 (72,6%)	HF	-	<i>Robber</i>
<b>Bombini</b>					
<i>Bombus</i> spp.	Nectar	3 (0,10%)	LF	100% (4)	<i>Occasional pollinator</i>
<b>Centridini</b>					
<i>Centris scopipes</i>	Nectar	5 (0,17%)	LF	80% (4)	<i>Occasional pollinator</i>
<i>Epicharis flava</i>	Nectar	8 (0,3%)	F	22% (9)	<i>Occasional pollinator</i>
<b>Euglossini</b>					
<i>Eulaema nigrita</i>	Nectar	2 (0,07%)	LF	0% (2)	<i>Neutral visitor</i>
<i>Euglossa</i> sp. 1	Nectar	1 (0,03%)	LF	-	<i>Neutral visitor</i>
<b>Meliponini</b>					
<i>Melipona</i> spp.	Nectar	14 (0,5%)	LF	-	<i>Neutral visitor</i>
<i>Paratrigona</i> spp.	Nectar/Pollen	38 (1,3%)	F	-	<i>Robber</i>
<i>Tetragona</i> spp.	Nectar/Pollen	19 (0,6%)	LF	-	<i>Robber</i>
<i>Tetragonisca</i> spp.	Nectar/Pollen	26 (0,8%)	LF	-	<i>Robber</i>
<i>Trigona</i> spp.	Nectar/Pollen	572 (19,4%)	HF	-	<i>Robber</i>
<b>Oxaeini</b>					
<i>Oxaea austera</i>	Nectar	11 (0,37%)	F	0% (2)	<i>Nectar robber</i>
<i>Oxaea flavescens</i>	Nectar	2 (0,07%)	LF	0% (1)	<i>Nectar robber</i>
<b>Xylocopini</b>					
<i>X. frontalis</i>	Nectar	62 (2,1%)	HF	82% (102)	<i>Effective pollinator</i>
<i>X. grisescens</i>	Nectar	4 (0,1%)	LF	100% (10)	<i>Occasional pollinator</i>
<i>X. suspecta</i>	Nectar	42(1,4%)	HF	44% (66)	<i>Occasional pollinator</i>

### 3.2 Functional groups density and natural pollination

We employed generalized models to analyze the bee diversity and visitation rates on natural fruit set within the study areas. The selection process led to the identification of the adjusted model, which excluded diversity (Shannon-Wiener Index) and all-pollinators visitation rates among the variables, demonstrating the most robust fit (AIC: -25.075) when compared to the full model (AIC: -22.183) and the null model (AIC: -12.465). The discrepancy between the adjusted model and the null model demonstrates once again the relevance of biotic pollination in passion fruit crops. The adjusted model showed a significant and positive impact of *Xylocopa frontalis* (*Effective pollinator*) visitation rate on the natural fruit set of passion fruit (Chisq=6.221, p=0.0126) (Figure 11a). Conversely, *Xylocopa*

*suspecta* (Occasional pollinator) exhibited no significant correlation with the model's outcomes.

Interestingly, the *Trigona* genus (Robbers) showed a significant and slightly positive effect on natural pollination (Chisq=4.296,  $p=0.0382$ ) (Figure 11b) while the *A. mellifera* (Robbers) visitation rate had a negative effect on natural fruit set (Chisq=5.035,  $p=0.024$ ), as the visitation rate of all-robbber bees (Chisq=5.655,  $p=0.0174$ ) (Figure 11c and Figure 11d). Based on our results, we observed that *Apis mellifera* bees carry the negative effect of robbers on yellow passion fruit production.



**Figure 11.** Percentage of natural fruit set of passion fruit orchards according to the abundance of bee's in *Triângulo Mineiro* region, Brazilian savanna. (a) *Xylocopa frontalis*, (b) *Trigona* genus, (c) *Apis mellifera*, (d) All robbers combine.

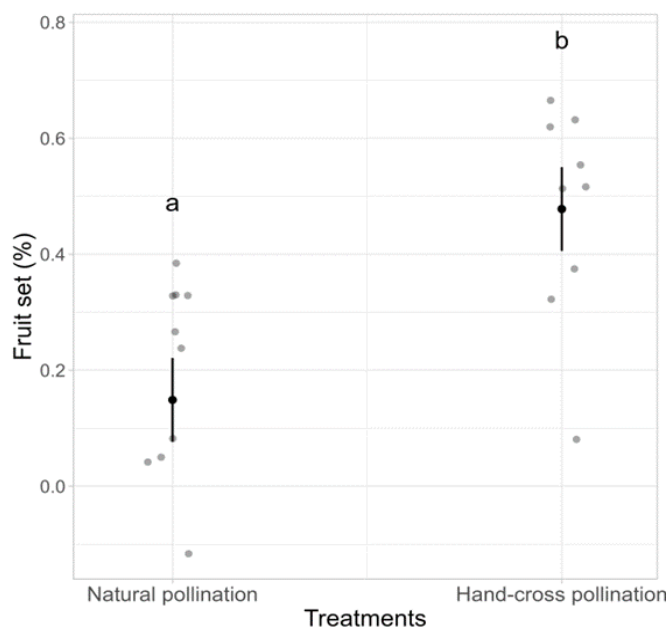
### 3.2 Comparing hand-cross and natural pollination in HDP and LDP areas

The hand-cross pollination treatment resulted in a higher fruit set compared to natural pollination (hand-cross pollination: 47.8%, natural pollination: 14.9%) ( $F = 45.811$ ,  $df = 1,18$ ,  $p = 2.429e-06$ ) (Figure 12). The hand-cross pollination treatment varied from 28% to 60% in the HPD areas and between 38% and 66% in the LPD areas (Table 3). Natural pollination varied from 2% to 4% in the HPD areas and between 16% and 34% in the LPD areas, demonstrating a wider difference between the deficit categories, as the activity of pollinators is the predominant factor influencing the variations in natural pollination. The areas with High Pollination Deficit (HPD) were MCJ02, MCJ05, MCJ07, MCJ09, MCJ10, while the areas with Low Pollination Deficit (LPD) were MCJ03, MCJ04, MCJ06, MCJ08 and MCJ11, indicating the occurrence of pollination deficit in half of the orchards. (Figure 13).

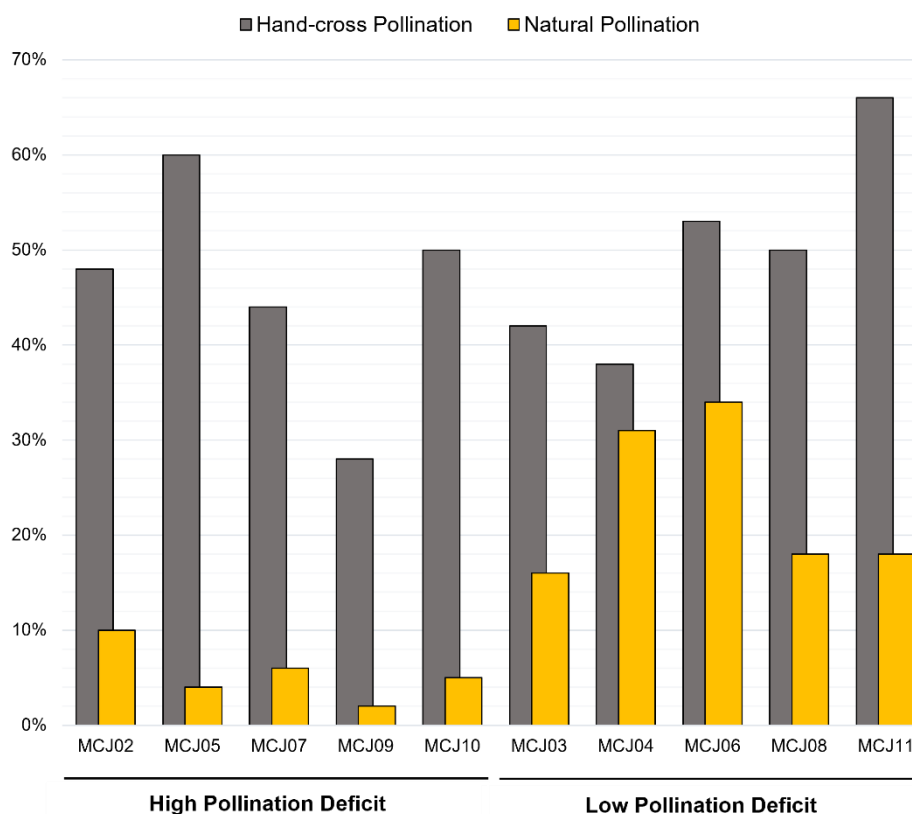
Moreover, we observed a significant difference in fruit set between the deficit categories ( $F = 12.443$ ,  $df = 1,8$ ,  $p = 0.007761$ ). LPD areas exhibited a fruit set until five times greater (Figure 14a). Moreover, the density of *Xylocopa frontalis* also was significant higher in LPD ( $F = 6.8085$ ,  $df = 1,8$ ,  $p = 0.03116$ ) (Figure 14b).

**Table 3.** Proportion of fruit set treatments, pollination deficit rank for each orchard of passion fruit in Triângulo Mineiro region, Minas Gerais State, Brazil.

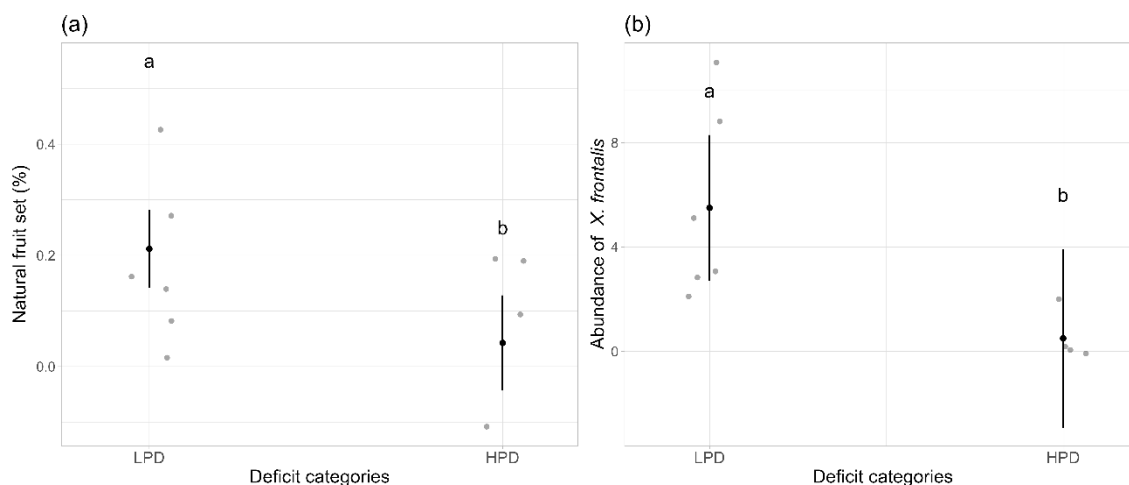
Orchards	Fruit set by treatment %		Deficit rank
	Hand-cross pollination	Natural Pollination	
MCJ2	48	10	HPD
MCJ3	42	16	LPD
MCJ4	38	31	LPD
MCJ5	60	4	HPD
MCJ6	52	34	LPD
MCJ7	44	6	HPD
MCJ8	50	18	LPD
MCJ9	28	2	HPD
MCJ10	50	5	HPD
MCJ11	66	18	LPD



**Figure 12.** Proportion of fruit set formation between pollination treatments, natural and hand-cross pollination, in passion fruit orchards in Triângulo Mineiro region, Minas Gerais, Brazil.



**Figure 13.** Variation of fruit set formation between pollination treatments, natural and hand-cross pollination, in passion fruit orchards in Triângulo Mineiro region, Minas Gerais, Brazil.

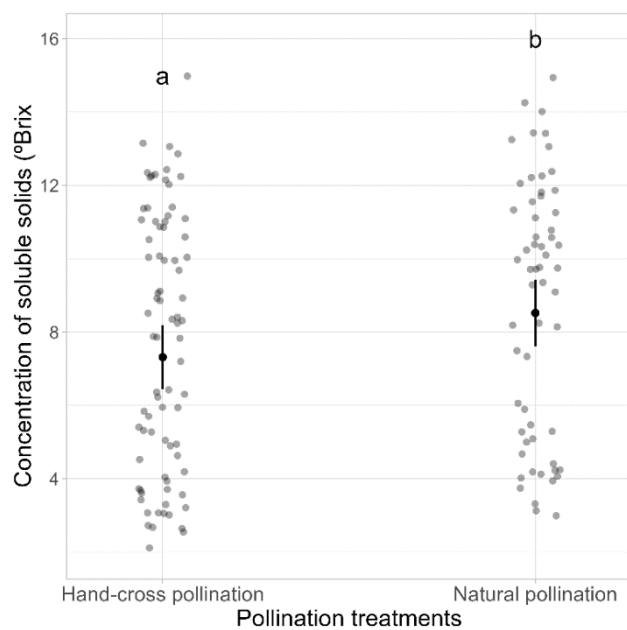


**Figure 14.** Differences in deficit categories of pollination according to (a) proportion of natural fruit set and (b) Abundance of *Xylocopa frontalis* in Triângulo Mineiro region, Minas Gerais State, Brazil.

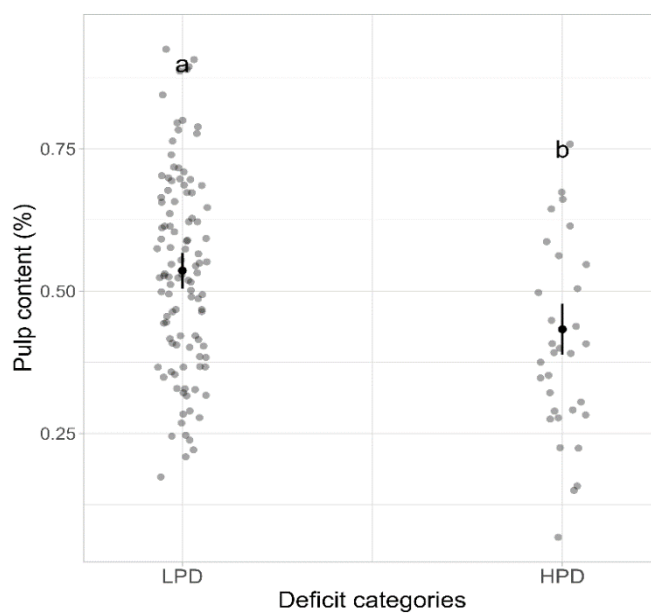
For the quality of the fruits, we observed that the LPD areas presented more balanced pulp content averages among treatments, with higher values for natural pollination (hand-cross pollination: 72.46g (53%), natural pollination: 75.23 (54%)). On the other hand, the HPD areas produced fruits with higher weight in hand-cross pollination (hand-cross pollination: 90.61g (46%), natural pollination: 50.27g (44%)). Significant differences were observed only across deficit categories ( $F = 16.3183$ ,  $df = 1, 136$ ,  $p\text{-value} = 8.908e-05$ ), while no differences were found among pollination treatments ( $F = 0.1676$ ,  $df = 1, 136$ ,  $p\text{-value} = 0.6829$ ), demonstrating an effect of the area on this variable and not of the treatments. The LPD areas exhibited 10% higher pulp content compared to HPD (Figure 15).

In a different way, the concentration of soluble solids in the pulp (brix<sup>o</sup>) was higher in both categories in natural pollination. Also, the LPD areas presented a bigger concentration of soluble solids in the pulp (LPD: hand-cross pollination: 8.39g, natural pollination: 9.15g; HPD: hand-cross pollination: 6.71g, natural pollination: 7.77g). We found significantly different between the pollination treatments ( $F = 3.9866$ ,  $df = 1, 136$ ,  $p\text{-value} = 0.04786$ ), but no differences between deficit categories ( $F = 2.2855$ ,  $df = 1, 136$ ,  $p\text{-value} = 0.13291$ ). The concentration of soluble solids, with represent the sweetness of the pulp, showed fruits 1.2<sup>o</sup> brix higher in natural pollination than hand-cross pollination (Figure 16), emphasizing

that differently of pulp content the pollinators can exchange better pollen amount than the hand-cross pollination.



**Figure 16.** Concentration of soluble solids (°Brix) between pollination treatments in Triângulo Mineiro region, Minas Gerais State, Brazil.



**Figure 15.** Proportion of pulp content in passion fruit according to deficit categories in Triângulo Mineiro region, Minas Gerais State, Brazil.



### 3.3 Land use coverage in the vicinity of passion fruit orchards

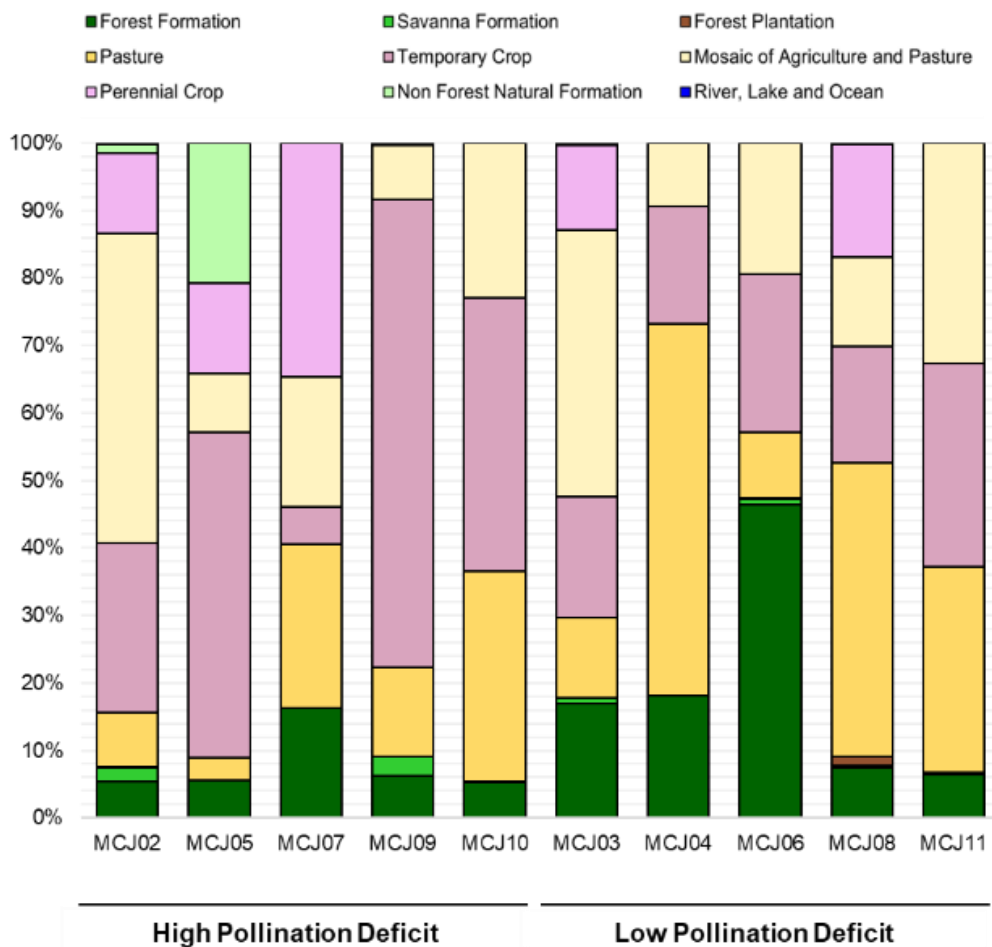
Examining the land use coverage in the vicinity of the orchards, we observed that the LPD areas, considered as a more balanced production system, within 1km and 4km ratios have a higher coverage of natural areas when compared to HPD, in almost all this refuges (e.g., forest formation in 1km: LPD. 16,76%, HPD. 8,32%). We also observed in LPD a larger coverage with lands characterized by moderate soil use, such as pastures (e.g., pasture in 1km: LPD 26.41%, HPD 17.99%). On the other hand, the high pollination deficit areas in both ratios had more coverage of intense soil use (e.g., temporary crop in 1km: LPD. 21,83%, HPD. 40,79%) (Table 4) (Figure 17).

We conducted an analysis using generalized models to investigate the impact of land use coverage on natural fruit set within the studied areas, both within one and four-kilometers radius from the passion fruit orchards. The results of the model showed a significant positive association between natural fruit set and the coverage of moderate land use within one-kilometer buffer surrounding the orchards (Chisq=4.8054,  $p=0.02837$ ), as illustrate in Figure 18a. The same one-kilometer buffer also exhibited a strong negative effect of intense land use in the natural fruit set in passion fruit (Chisq=15.9271,  $p=6.583e-05$ ), as represented in Figure 18b, but the forest formation exhibited no statistically significant effect in the model analysis. The model involving a four-kilometer buffer did not show any significant relationship with the natural fruit set of passion fruit.

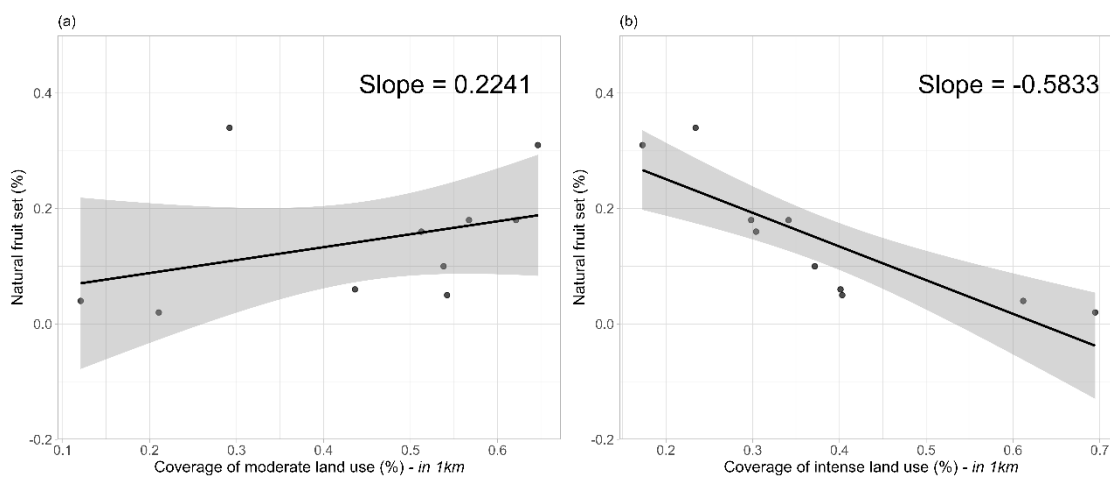
We also investigated whether the spatial pattern of land use intensity in the vicinity of passion fruit in 1km radius interfered with the functional groups through the abundance of highly frequent species in the observations. *Xylocopa frontalis* demonstrated a significant and strongly positive correlation with vegetation cover (Chisq= 8.9942,  $p=0.002708$ ), and a strongly negative correlation with intense soil use (Chisq= 16.884,  $p= 3.975e-05$ ) (Figure 19a). In contrast, *Apis mellifera* demonstrated a strong negative effect with vegetation cover (Chisq= 3.8951,  $p= 0.04843$ ) and a high positive correlation with the increase in intense soil use (Chisq= 7.7706,  $p= 0.00531$ ) (Figure 19b). The *Xylocopa suspecta* and the *Trigona genus* did not present any correlation, neither with vegetation cover, moderate soil use, or intense soil use.

**Table 4.** Proportion of land use cover of vegetation, moderate soil use and intense soil use according to deficit categories in Triângulo Mineiro region, Minas Gerais state, Brazil.

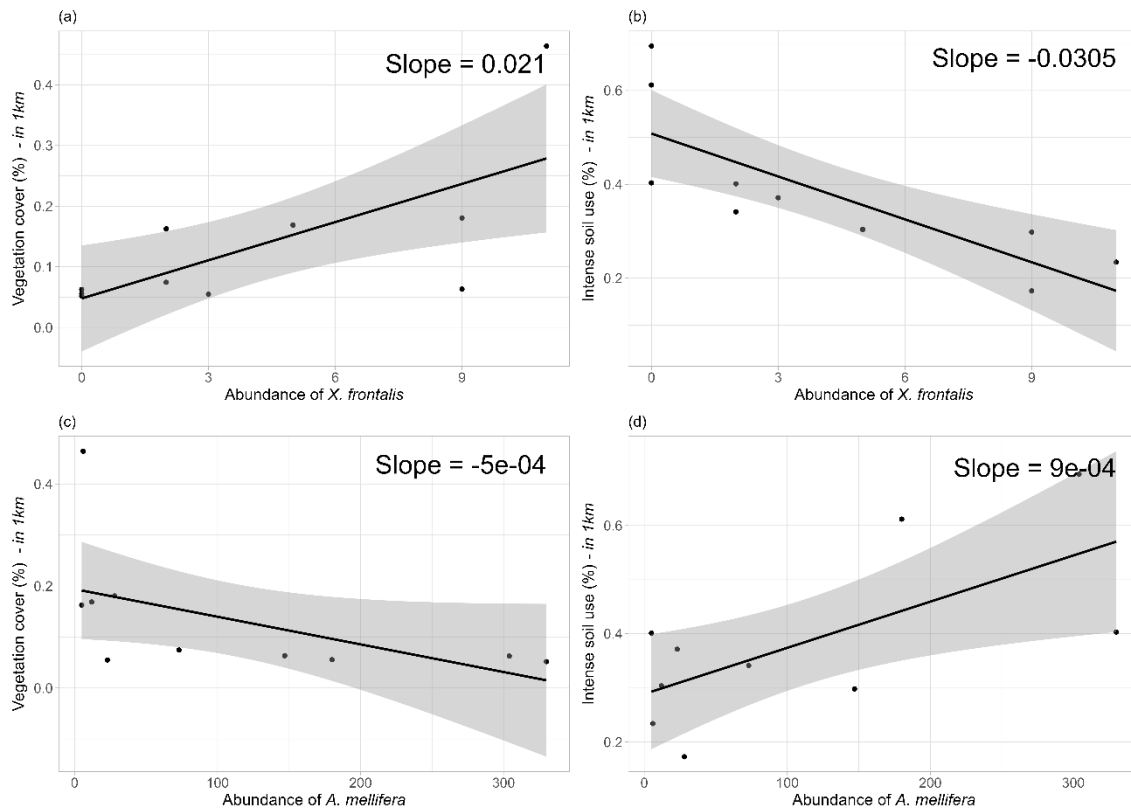
<b>Classes of land use in 1 kilometer buffer ratio</b>	<b>Deficit categories</b>	
	<b>LPD</b>	<b>HPD</b>
<i>Vegetation cover</i>		
Forest formation	16,76%	8,32%
Savanna	0,72%	0,77%
Non-forest natural formation	0,61%	5,27%
<i>Moderate soil use</i>		
Mosaic of agriculture and pasture	26,54%	14,74%
Pasture	26,41%	17,99%
<i>Intense soil use</i>		
Perennial crops	6,88%	11,98%
Forest plantation	0,50%	0%
Temporary crops	21,83%	40,79%
<b>in 4 kilometer buffer ratio</b>	<b>LPD</b>	<b>HPD</b>
<i>Vegetation cover</i>		
Forest formation	15,22%	11,94%
Savanna	2,10%	1,66%
Non-forest natural formation	0,43%	0,57%
<i>Moderate soil use</i>		
Mosaic of agriculture and pasture	22,02%	23,85%
Pasture	25,34%	21,40%
<i>Intense soil use</i>		
Perennial crop	7,03%	7,68%
Forest plantation	0,66%	0,29%
Temporary crop	24,82%	29,36%



**Figure 17.** Variation of land cover use between pollination deficit categories in passion fruit orchards in Triângulo Mineiro region, Minas Gerais, Brazil.



**Figure 18.** Relation between the proportion of natural fruit set and the coverage of land use in the vicinity of passion fruit orchards. (a) Moderate land use, encompassing pastures and mosaic of agriculture and pasture, (b) Intense land use, encompassing perennial and temporary crops.



**Figure 19.** Relation between the percentage of vegetation cover and intense soil use in the vicinity of passion fruit orchards and the abundance of *Xylocopa frontalis* (a-b) and *Apis mellifera* (c-d).

#### 4. Discussion

Our study showed that intense soil use in the vicinity of crops affects negatively the native bees (pollinators or robbers) and the fruit set of passion fruit. We also verified that the low pollination deficit (LPD) areas exhibited a fruit set until five times greater and higher pulp content than high pollination deficit (HPD).

Quantitative comparisons of different behaviors and foraging strategies between pollinators have been used for a long time to evaluate the effects of pollinators on crops (Primark, 1975). We applied this perception to refine the classification of passion fruit pollinators in Brazilian savannas according to their frequency of occurrence (FO) and effectiveness in pollen deposition (EPD).

Only the largest bees, *X. frontalis* and *X. grisescens*, *Bombus* spp. and *Centris scopipes*, showed enough EPD to their thorax when visiting the flowers, being considered possible effective pollinators. *Xylocopa suspecta* and *Epicharis flava* are slightly smaller bees and presented contact with the anthers of the flowers in less than half of their visits. Therefore, also considering the FO, the only bee specie categorized as an effective pollinator of passion fruit in this region is *Xylocopa frontalis*.

All robbers were social bees, the exotic bee *A. mellifera* and the stingless bees from the genus *Trigona*, *Paratrigona*, *Tetragona* and *Tetragonisca*. While collecting pollen or nectar, from legitimate or illegitimate visits, these bees could not successfully pollinate the flowers by their small size and be a treat to the yield due to its recruitment behavior performed to food resource collection (Sazima and Sazima 1989). Robbers are frequently described as cheaters in plant-pollinator mutualism interactions because they obtain a reward without providing a pollination service, but this behavior must not be directly correlated with decreased productivity (Maloof and Inouye, 2000).

Analyzing the influence of visitation rates of the most frequent bees on the fruit set of passion fruit, we confirm that only *Xylocopa frontalis* had a significantly and positive effect on the fruit set. *Xylocopa suspecta*, despite its high frequency in passion fruit crops, was not present significant effect. Melo et al. (2014) demonstrated in single-visit pollination experiments that *X. suspecta* did not produce fruit in passion fruit, as it was observed in single-visits of *X. frontalis*. Similar observations were made for *X. cearensis* in the Caatinga, since this is also a smaller *Xylocopa* bee (Siqueira et al. 2014).

When collecting nectar, *Xylocopa* bees demonstrate a rotational behavior on the flower, which, combined with the necessary size for flowers anatomy, maximizes the chances of pollen being deposited on all stigmas of the visited flowers. Moreover, the quantity of fruit formed in passion fruit depends on the number of pollinated stigmas, being 36% higher when all three stigmas of the flower are pollinated by *X. frontalis* (Melo et al., 2014). Another relevant factor in the formation of these fruits is the number of visits by *X. frontalis* per flower. When receiving at least two visits, more than 50% of the fruit is formed (Martarello et al., 2021).

Despite not representing a significant number of individuals in the study region, *Xylocopa grisescens* is also an effective pollinator in passion fruit (Yamamoto *et al.*, 2012; Martins *et al.*, 2014), as they have the same size and collecting behavior as *X. frontalis*. This species was the most frequent pollinator observed in Caatinga in studies with yellow passion fruit and had a higher number of nests than *X. frontalis* (Martins *et al.*, 2014, Viana *et al.* 2014).

In Brazilian savanna both species are present, but *X. frontalis* is more frequent (Yamamoto *et al.*, 2012; Junqueira *et al.*, 2013). During experiments performed with passion fruit in Atlantic Forest, *X. grisescens* was not observed in either Rio de Janeiro state (Gaglianone *et al.*, 2014) or Paraná state (Melo *et al.* 2014), while *X. frontalis* was present in both. These reports are in accordance with the distribution observed for the species (Moure, 2012). *Xylocopa grisescens* has a smaller distribution than *X. frontalis*, overlapping only in the Caatinga biome and the limits of the Brazilian savanna, while *X. frontalis* is distributed throughout the Neotropical region.

Our study also showed the social stingless bees from the genus *Trigona*, presented a significant and positive effect on the fruit set. As the *Trigona* bees, *Trigona spinipes* or *Trigona hyalinata*, could not pollinate the flowers, we assumed that this is a result of an indirect effect on pollination. This result highlights the balance between the natural diversity of flower visitors in the passion fruit orchards. Moreover, the visits of all robbers together present a negative effect on the fruit set of passion fruit, due the behaviors performed by *Apis mellifera*. We observed that the exotic honeybees are highly detrimental to passion fruit cultivation, being the main responsible for the strongly negative interaction between fruit set and robbers.

*Apis mellifera* was highly abundant in this study, with three times more individuals than the *Trigona* bees. This bee is exotic and voracious in resource collection, and it is observed collecting both pollen and nectar. However, it is commonly seen collecting pollen even during the anthesis of the flowers. The pollen removal occurs quickly, needing 10 seconds to remove more than 20% of pollen from an anther (Gaglianone *et al.* 2014). This behavior is extremely harmful to the cultivation, as these quick pollen removal processes, known as consumptive emasculation, directly reduce the plant's opportunities for successful mating. With

that, *Apis mellifera* decreases the availability of pollen to the effective pollinators, such as *Xylocopa frontalis* (Siqueira *et al.* 2009; Yamamoto *et al.* 2012, Junqueira and Augusto, 2017), and the possibility of hand-cross pollination (Freitas and Oliveira Filho, 2001).

The stingless bees from the *Trigona* genus, despite not being effective pollinators of passion fruit due to their small size, do not present a negative effect on pollination. This is probably due to their lower frequency in pollen collection at the beginning of the flower anthesis and the higher frequency of nectar collection observed in all orchards. Although mechanical injuries caused by flower perforation did not demonstrate any detriment to the formation of passion fruit (Silva, 1997; Melo *et al.*, 2014), the bees robbing nectar decrease the visit time of *Xylocopa* bees per flower (Sazima and Sazima, 1989), indirectly leading to a higher visitation rate in passion fruit orchards. Additionally, the *Trigona* genus is aggressive and attacks *Xylocopa* bees on passion fruit flowers (Sazima and Sazima, 1989), which increases the frequency of pollinator transfers from flower to flower through interspecific behavioral interactions and increases the per-visit effectiveness (Greenleaf and Kremen, 2006). Both behaviors corroborate with the data presented in this study that these bees do not interfere negatively with productivity. However, it is important to note that when collecting pollen, these bees cause the same effect as *Apis mellifera*, reducing the amount of pollen available in the orchards (Silva *et al.* 2014).

The fruit set and the amount of pulp produced in passion fruit, regardless of manual or natural pollination, are related to pollination deficits in the areas, while the sweetness of the pulp is higher in natural pollination treatments. High dependence on pollination is considered the main cause of low yield for passion fruit orchards. The fruit set by natural pollination was lower than the hand-cross pollination in all orchards, indicating a pollen limitation under natural pollination. All around the country the hand-cross pollination usually presents this same outcome (Presidente Prudente, SP: Yamashiro, 1981; Campinas, SP: Sazima and Sazima, 1989; Londrina, SC: Neves *et al.*, 1999; Juazeiro, BA: Bos *et al.*, 2007; Araguari and Uberlândia. MG: Yamamoto *et al.*, 2012, Junqueira *et al.*, 2016; Paço do Lumiar, MA: Martins *et al.*, 2014). This difference demonstrates that pollination acts as a limiting factor for passion fruit production in a larger scale and that bee-

friendly strategies must be implemented in the fields to improve pollination and increase passion fruit yield. In many areas, farmers are achieving below maximum yields due to insufficient pollen deposition, which can have significant impacts on crop production and market value.

Evaluating the quality of the fruits formed in the studied passion fruit orchards, we observed that the pulp content varied significantly between the established deficit categories for the crops. The LPD areas presented a higher concentration of pulp in the fruits. Similar values were observed in other studies for the hybrid studied (Moura *et al.*, 2020 and Cobra *et al.*, 2015). On the other hand, the soluble solids, which represent the sugar concentration in the fruit pulp, were significantly related to natural pollination in the orchards. Even though soluble solids values were higher in natural pollination, the concentration of these sugars in both treatments was lower than that observed by Moura *et al.* (2020) and Cobra *et al.* (2015) with an average of 12.25° brix and 13.1° brix, respectively.

The greater fruit set and the abundance of the two main bee species (*X. frontalis* and *A. mellifera*) that influence the fruit set are related to the surroundings of passion fruit crops. Areas of moderate soil use, as pastures, are positively related to a greater fruit set, while areas of intense soil use, as soybeans crops, are negatively influence this fruit set. Moreover, vegetation cover is positively correlated with *Xylocopa frontalis* and negatively correlated with *Apis mellifera*, while intense soil use presented the opposite behavior, being positive for *Apis mellifera* and negative for *Xylocopa frontalis*.

In contrast, *Apis mellifera* is more abundant in areas with higher coverage of intense soil use and less frequent in areas with higher vegetation cover. Factors related to this high adaptability to areas with poorly distributed resources and mass flowering are the eusocial behavior of these bees, which collect and store resources in groups for times of lower availability. Due to these behaviors and their small size, these species are highly frequent in soybean crops and are well-suited to areas where this crop is produced.

In all South America, soybean expansion has been associated with high rates of deforestation and biodiversity loss, resulting in a high environmental cost (Aizen *et al.*, 2019). This study observed that intense soil use, mainly represented by soybean cultivation in this region, also favors the expansion of the distribution of



*Apis mellifera*, while decreasing the distribution of *Xylocopa frontalis* and the productivity of yellow passion fruit.

In this study, we found that the vegetation cover within a one-kilometer radius of the crop is relevant to the rates of natural fruit set, but no significant relationship was observed with the four-kilometer ratio, despite the large flight radius of these bees (Camillo, 2003; Freitas and Oliveira-Filho, 2001).

Areas classified as moderate land use are positive related with the passion fruit yield and are composed of large pastures and small patches of varied soil use throughout the year. These environments, especially in Brazilian savannas, function as a large area for soil rest and regeneration. Moderate disturbance areas can enhance the density of pollinators by expanding the cover of herbaceous plant species, thereby increasing nectar and pollen availability for pollinators (Winfrey *et al.* 2007) and the abundance of nesting resources within wooden substrates in pastures (Steffan-Dewenter and Leschke, 2003), that may also facilitate the nesting and dispersal of those bees.

Intensive land use areas, including perennial and temporary crops, have been found to be negatively related to passion fruit set. These areas are typically characterized by large monocultures, particularly of soybean, which has increasingly used more pesticides since the 1990s (Coupe e Capel, 2015). The exposure to pesticides and chemical products used in agricultural lands have negative impacts on insects, especially those associated with pollinator-dependent crops (Kopit and Pitts-Singer, 2018), forming a barrier to bee foraging and by consequence, directly affecting the yield of passion fruit.

Passion fruit crops are under different pressures due to land cover changes and forest loss induced by anthropogenic factors. Furthermore, these crops are highly dependent on large size bees, particularly bees of the genus *Xylocopa* and in the region studied the *X. frontalis*. Moreover, the fruit set of passion fruit is highly negative affected by the exotic robbers, *Apis mellifera*. Junqueira *et al.* (2013) demonstrated the possibility of reducing the visitation rates of *Apis mellifera* in fruit crops by enhancing the availability of nests for carpenter bees once these nests can increase their population density and be an effective strategy to reduce the visitation rates of *Apis mellifera*. In such wise, planning crops and management strategies, away from areas of intensive soil use and close to pastures and natural

areas is a bee-friendly practice that can favor passion fruit yield and help maintain the presence and density of *Xylocopa* bees in the region and by that, in the passion fruit orchards.

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## CAPÍTULO 2

**Nesting behavior and phenology of *Xylocopa frontalis* Olivier and *Xylocopa grisea* Lepeletier (Apidae: Xylocopini) in Brazilian savanna**



## Resumo

As abelhas carpinteiras, ou mamangavas de toco, pertencem exclusivamente ao gênero *Xylocopa*, dentro da tribo Xylocopini, que representa aproximadamente 30% de todas as espécies de abelhas e é um grupo distinto de abelhas que constroem ninhos acima do solo. *Xylocopa frontalis* e *Xylocopa grisescens* são duas espécies simpátricas e intimamente relacionadas com características bionômicas e preferências de substrato semelhantes. Neste estudo, avaliamos os comportamentos de nidificação e comparamos a longevidade e reutilização de ninhos para essas espécies. Também analisamos os padrões de fenologia da produção de crias para examinar o tempo e as tendências dessas espécies ao longo de três anos consecutivos no cerrado brasileiro. *Xylocopa grisescens* teve ninhos mais duradouros e reutilizou mais frequentemente os ninhos de bambu, enquanto *X. frontalis* teve ciclos de ninho mais curtos e menor reutilização. A atividade anual de células de cria de ambas as espécies não foi uniformemente distribuída ao longo do ano. *Xylocopa frontalis* produziu 73% de sua prole na estação chuvosa, enquanto *X. grisescens* produziu 60% de sua prole no meio da estação seca. Ambas as espécies apresentaram tendência a serem bi ou multivoltinas, e o pico de produção de células de cria entre as espécies, de acordo com a sazonalidade, mostrou que o tempo de atividade de criação entre as espécies está correlacionado com a sazonalidade e influenciado por fatores ambientais. Com base no exposto, é importante desenvolver diferentes estratégias de manejo para garantir a disponibilidade de recursos alimentares nos momentos apropriados e manter ambas as populações em novos ambientes para o manejo dessas espécies no cerrado brasileiro.

**Palavras-chave:** Abelha carpinteira, mamangava de toco, áreas de criação, longevidade, reuso, fenologia, cerrado.

## Abstract

Large carpenter bees belong exclusively to the genus *Xylocopa* within the tribe Xylocopini, which accounts for approximately 30% of all bee species and is a distinguished group of above-ground nesting bees. *Xylocopa frontalis* and *Xylocopa grisescens* are two sympatric and closely related species with similar bionomic characteristics and substrate preferences. In this study, we evaluated the nesting behaviors and compared nest longevity and reuse for these species. We also analyzed the phenology patterns of brood production to examine the timing and tendencies of these species over three consecutive years in the Brazilian savanna. *Xylocopa grisescens* had longer-lasting nests and reused bamboo trap-nests more frequently, while *X. frontalis* had shorter nest cycles and lower reuse. The annual brood cell activity of both species was not uniformly distributed throughout the year. *Xylocopa frontalis* produced 73% of its offspring in the rainy season, while *X. grisescens* produced 60% of its offspring in the middle of the dry season. Both species exhibited a tendency to be bi or multivoltine, and the peak of brood cell production between the species, according to seasonality, showed that the timing of brood activity between the species is correlated to seasonality and influenced by environmental factors. Based on the above, it is important to develop different management strategies to ensure the availability of food resources at the appropriate times and maintain both populations in new environments for the management of these species in the Brazilian savanna.

**Keywords:** Carpenter bees, breeding sites, longevity, reuse, phenology, Brazilian savanna.

## 1. Introduction

The large carpenter bees belong exclusively to the genus *Xylocopa* within the tribe *Xylocopini*. They exhibit a predominant distribution in tropical and subtropical regions, but can also be found in temperate areas, although they are less common in those regions (Hurd and Moure 1963, He and Zhu 2018). It is believed that the clade most likely originated in the Oriental-Palearctic region and subsequently underwent dispersal to all continents, excluding Antarctica, through independent events in the evolutionary history of the tribe (Mérida-Rivas et al. 2022). The Neotropical species of *Xylocopa* have undergone significant diversification, particularly in the Caribbean islands and South America, with 49 species belonging to the highly diverse subgenus *Neoxylocopa*. Within this subgenus, the region encompassing Mexico and Mesoamerica has identified 9 species (Mérida-Rivas et al. 2022), while Brazil alone supports 20 species (Marchi and Alves-dos-Santos 2013).

*Xylocopa* belongs to the distinguished group of above-ground nesting bees, that account for approximately 30% of all bee's species (Vaughan et al. 2014). Carpenter bees are named as such because the adult females of many species excavate nests in dead wood, including soft or rotten wood, floral scapes, or bamboo cavities (Camillo and Garófalo 1982, Camillo et al. 1986, Silveira 2002, Schlindwein et al. 2003), forming complex and branched galleries. Excavating this wood demands high energy costs from the bees (Ostwald et al. 2021), and these costs favor the reuse of existing nests, increased tolerance, and cooperation of other individuals (Peso and Richards 2010)

The main characteristic of *Xylocopa* nests, which is also common to the relictual *Manueliini* tribe, the basal group, and the distal group *Ceratinini* within *Xylocopidae*, is the presence of excavated sawdust partitions inside the nest (Rehan et al. 2012). These partitions are compacted and mixed with saliva by the females to protect the offspring against dehydration or excessive humidity (Gerling et al. 1989). Each brood cell is individualized and contains a bell-shaped mass of pollen and an egg (Marchi and Melo 2010, Pereira and Garófalo 2010, Lucia et al. 2017). These cells are constructed sequentially and repeatedly from the back of the nest to the front (Flores-Prado et al. 2010).

Renowned for their large size and high flight capabilities, these bees utilize a vast foraging area, exhibit floral constancy, and engage in buzz behavior enabling them to efficiently gather limited resources from the environment. This remarkable adaptation to resource constraints, combined with their extended lifespan, overlapping generations, and parental care, positions these bees as an intriguing study model for investigating primitive bee societies (Buchmann and Minckley 2019).

The cavity-nesting bees are good model to study the bionomic characteristics, as substrate preferences, nest architecture, sexual ratio and foraging behaviors. In Brazil, the nesting behaviors were described for: *X. suspecta*, *X. frontalis* e *X. grisescens* (Camillo and Garófalo 1982, Camillo et al. 1986, Camillo and Garofalo 1989, Viana et al. 2001, Marchi and Melo 2010, Pereira and Garófalo 2010, Chaves-Alves et al. 2011, Bernardino and Gaglianone 2013, Martins et al. 2014, Pedroso et al. 2021, Souza et al. 2021), *X. abbreviata* (Ramalho, Batista and Silva 2004), *X. cearencis* (Viana, Kleinert and Silva 2002, Silva et al. 2019, Souza et al. 2021), *X. artifex* (Silveira 2002), *X. subcyanea* (Silva and Viana 2002, Gimenes et al. 2006) e *X. ordinaria* (Bernardino and Gaglianone 2013).

In Brazilian savanna (Minas Gerais state, Brazil) nests of *X. suspecta*, *X. hirsutissima*, *X. subcyanea*, *X. suspecta*, *X. frontalis*, and *X. grisescens* were documented in wooden fence posts of *Sphatodea campanulate* while only *X. frontalis*, *X. grisescens* and *X. suspecta* was recorded in bamboo trap-nests (Chaves-Alves et al. 2011, Pedroso et al. 2021).

These two sympatric and closely related species, *X. frontalis* and *X. grisescens*, that cohabit the same breeding sites and share similarities in: size (30 to 36mm for *X. frontalis* and 30mm for *X. grisescens*) (Marchi and Alves-dos-Santos 2013), nest diameter in bamboo trap-nests (in average 20mm for *X. frontalis* and 18mm for *X. grisescens*) (Chaves-Alves et al. 2011), and development from egg to imago (average of 45-65 days for both species) (Camillo and Garófalo 1982). After the emergence of the brood cell, these newly hatched bees remain in their natal nest for approximately 30 days to allow the circulation of hemolymph within their wing veins and to mature physiologically (Camillo and Garofalo 1989). After this period, the males disperse from the natal nest. The females can also disperse and found

their nest solitarily or stayed in the nest, associated to mother and/or sisters (Camillo et al. 1986, Camillo and Garofalo 1989).

As demonstrated, the nesting behaviors of these species have been extensively studied in Brazil, primarily due to their importance as pollinators of native plant species in such a diverse region as the Neotropics. They also hold significant economic value in Brazil, as they are effective pollinators of various cultivated plants, with notable examples including the yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa* Deg.) (Junqueira and Augusto 2017) and the Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.) (Cavalcante et al. 2012).

*Xylocopa frontalis* and *X. grisescens* exhibit low connectivity and high specialization in the types of pollen collected for provisioning brood cells (Araújo et al. 2021). These different strategies allow for reduced competition for resources between these species, but differences in their use of other ecological resources related to their intranidal behavior have not been analyzed, which could affect their coexistence in the same environment. Considering that these are two potentially manageable species for pollination, understanding their ecological resource use strategies is essential for defining appropriate management strategies.

Our hypothesis is that these sympatric species do not completely overlap in their phenological cycles of brood cell production, and that these different strategies allow for reduced competition for resources throughout the year. Therefore, in this study, we evaluated the nesting behaviors and compared nest longevity and reuse for *Xylocopa frontalis* and *Xylocopa grisescens* in bamboo trap-nests. We also analyzed the phenology patterns of brood production to examine the timing and tendencies of these species over three consecutive years in the Brazilian savanna.

## **2. Material and Methods**

### *2.1. Studied areas and trap nests*

We conducted this study in two Brazilian savanna areas apart for 10 kilometers: Experimental Station of Água Limpa (ESAL) and Panga Ecological

Station (PES), located at Triangulo Mineiro region, Minas Gerais State, Brazil. The climate of the region is tropical, with two well-defined seasons: the rainy summer (October to March) and the dry winter (April to September) (Rosa et al., 1991).

Both areas are in Brazilian savanna, belong to the Federal University of Uberlândia and are used for distinct purposes. ESAL is a mixed system farm with 194.72 ha of pastures, crops and orchards (including yellow passion fruit, mango, pineapple, guava, tamarind and acerola) and 104 ha of natural savannah areas with remnants of cerrado, cerrado sensu stricto, and gallery forests (Neto, 2008). PES is a natural reserve with 403.85 ha predominantly characterized by savannah vegetation, encompassing a diverse range of plant formations (sparse cerrado, typical cerrado, and dense cerrado), and also gallery forest (Araújo and Schiavini, 1989).

We believe that by analyzing these two areas together, we can obtain a comprehensive understanding of the habitat complexity for the populations in the region. As described, these areas likely encompass a range of ecological features, such as vegetation types and microhabitat variations, which collectively contribute to the overall habitat complexity and represent the environmental conditions and factors that influence the species of interest.

The bee shelters, measuring 2.5 m in height and 1.5 m in length, consist of bamboo sticks closed at one end by the node. These bamboo sticks are organized inside 15 building bricks, usually with eight hollows, which are then placed on shelves to serve as trap-nests with approximately 120 nests per area with the diameter needed for *Xylocopa* bees (Figure 1).

## 2.2. Data collection

Monthly observations were conducted in the shelters located in both areas to gather data. Each nest was tagged, and each female bee was individually marked with paint and numerical target. Detailed records were maintained for each nest, including the number of females, males, and cells production.

These individual marks allowed us to evaluate the preferences in nest activation, as well as the nest's longevity, reproductive cycles, and nest reuse,

providing valuable perceptions into the patterns exhibited by both species in the trap-nests. We also conducted an analysis of the phenology patterns at brood production using monthly temporal data that allow us to examine the timing and trends of those species over three consecutive years: 2020, 2021, and 2022.



**Figure 1.** PER bee shelter with building bricks fulfill with bigger bamboo trap-nests, corresponding to the diameter needed for *Xylocopa* bees, in the left side.

### 2.3. Description of Parameters

#### 2.3.1. Species patterns:

*Trap-Nest occupation:* A new nest foundation was established either by the occupation of an empty nest by a female born in the shelter or by the observation of a new unmarked female in one of the bamboo trap-nests. The females leave the nest before beginning the production of brood cells.

*Nest foundation:* It was characterized by the initiation of provisioning of the first brood cell in a new nest by a female. This process was identified by the deposition of a homogenized mixture of pollen and nectar at the end of the brood cell or the observation of other subsequential phases of the process (e.g., oviposition or operculation).

*Reproductive cycles:* The period of cell production and immature developmental state inside the maternal nest, whether during nest foundation or in possible subsequent reuses.

*Nest longevity:* The longevity was presented only to nest in which had a cell production, and it was calculated from trap-nest occupation still the dispersion of nesting females.

*Nest reuse:* Is referred to the utilization, or reactivation, of a nest previously occupied by another female, following a period of nest vacancy.

### 2.3.2. Phenology pattern:

To access the cyclic patterns of brood cell production, we utilized the total number of cells in the studied shelters across the months of all 3 years of observation.

### 2.4. Data analysis

To compare the variations in individual nest patterns (longevity and reuse) between the species, we utilized a General Linear Mixed Model with gaussian family using the “lmer4” package. These models incorporated “year” and “bee shelter” as random factors to capture the intrinsic variability within each specie. As longevity and the number of reproductive cycles is highly correlated, we will analyze only the longevity of the nests.

We utilized circular statistics to investigate the phenology of the two species of *Xylocopa* studied using package “circular” and “mixtools”. The circumference representing the year was divided into 12 sectors, with the midpoints of each sector representing the months, as described by Morellato et al. (2010). This approach is particularly useful for phenological data, allowing us to evaluate seasonality for each specie.

First, we assessed the concentration of data around one or multiple points on the circle by calculating the mean resultant length (vector  $r$ ) for each species. Next,



we conducted Watson's goodness of fit test at a significance level of 0.05 to investigate if the data followed a von Mises distribution, which represents "circular normality." None of the species presented a uniform Von Mises distribution, making it more appropriate to use Rao's spacing test to evaluate if the data was uniformly distributed (Jammalamadaka & Sengupta, 2001). The maximum circular correlation coefficient of the function is 1, which represents that the data tends to cluster, and the minimum degree of aggregation of 0 indicates a lack of spatial dependence.

Accepting the uniformity of the samples, we observed the occurrence of peaks by analyzing the visual observations of the data. After that, we proceeded to investigate if the species had different median directions using the Watson-Wheeler test. This statistical test allowed us to investigate whether significant differences existed in the angular distribution of phenological activities between the species throughout the year.

Statistical analyses were performed using the software R (version 2023.03.1, R Foundation for Statistical Computing, Vienna, Austria).

### **3. Results**

#### *3.1 Occupation of trap-nest and effective production of nest*

During the three years of experiment, we observed 102 females *X. frontalis* occupying 69 trap-nest, but only in 38 of those trap-nests, 55%, we observed nest foundation, with a production of 93 brood cells. A total of 39 new individuals were observed on those nests, consisting of 31 females and 8 males, which represent 41% of emergences. Then, 58% of the offspring dispersed from the maternal nest before to be observed by us (Table 1). To *Xylocopa grisescens*, we observed 30 occupied trap-nests with 57 females. Only 50% of those occupations turn out to nest foundations (n=15), producing 52 brood cells. We observed 32 new individuals, including 22 females and 10 males, which represent 61,5% of total of brood cells (Table 1).

The data collected about reproductive cycles allowed us to observe multiple consecutive reproductive cycles in the nests. Both species exhibited nests with a

single reproductive cycle, characterized by one subsequential production of brood cells at a point in time (34 active nests for *X. frontalis*; 9 for *X. grisescens*), as well as continued nests, with up to four reproductive cycles performed by the founding female and later by one or more females present in the nest (4 active nests for *X. frontalis*; 7 for *X. grisescens*). The maximum number of consecutive reproductive cycles observed for both species was four. The longer nest of *X. frontalis* produced 9 brood cells (7 females and 1 observed male) over a span of 13 months, while the longest of *X. grisescens* produced 14 brood cells (8 females and 4 observed males) in 17 months. *Xylocopa grisescens* presented an average of 2.3 individuals per nest, while *X. frontalis* presented an average of 1.6 individuals per nest.

**Table 1.** Nesting patterns in Brazilian savanna bee shelters between 2020 and 2022. Minimum and maximum values per nest represent the total of production in all consecutive cycles per nest.

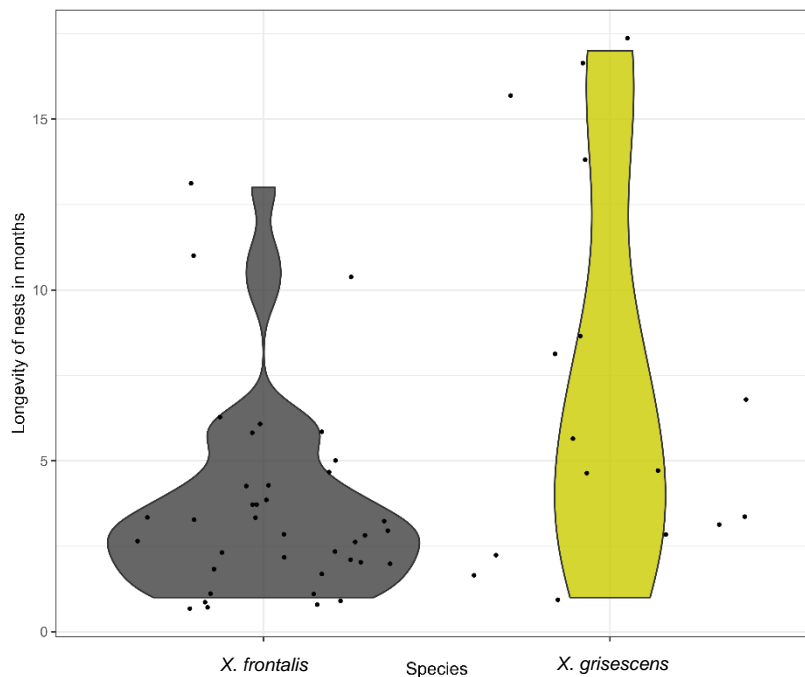
Species patterns	<i>X. frontalis</i>	<i>X. grisescens</i>	p (>Chisq)
Trap-nest occupation	69	30	-
Nest foundation	38 (55%)	15 (50%)	-
min-max per nest	1-8	1-8	-
Total females	102	57	-
min-max per nest	0-9	0-14	-
Offspring observed	39 (41%)	32(61%)	-
female	31	22	-
male	8	10	-
Nest longevity	3.63 ± 2.76	7.37 ± 5.63	0.00010 **
Nest reuse	34.2%	62.5%	0.0468 *

Nest longevity and reuse present respective standard errors. Coefficients significance of population patterns are Chisq: \* P ≤ 0.05, P ≤ \*\*0.001

Upon analyzing the reproductive traits exhibited in the nests of both species, significant differences were observed in nest's longevity (Chisq=10.815 p=0.0010) (Figure 3) and in the proportion of nest reuse (Chisq=3.953, p=0.0468). *Xylocopa grisescens* exhibited longer-lasting nests and reused these nests in 62% of the cases, whereas *X. frontalis* presented more nests, shorter cycles, and lower rates of nest reuse, with 34% (Table 1).

### 3.2 Brood cells production phenology patterns

The mean resultant length (vector  $r$ ) is a measure of degree of concentration around the mean direction. In this study, *X. grisescens* showed a moderate tendency for spatial aggregation but not an intense clustering ( $r = 0.361$ ). Similarly, *X. frontalis* showed a similar tendency for aggregation ( $r = 0.457$ ), although more intense than *X. grisescens*. The median of the data, which provides the likely date of the highest angle aggregation, corresponds to February for *X. frontalis*, at the end of the rainy season, and September for *X. grisescens*, at the end of the dry season in Brazilian savanna (Table 2, Figure 4) showing an influence of seasonality.



**Figure 3.** Nest longevity in *Xylocopa frontalis* and *X. grisescens*.

The annual brood activity pattern of both species was not uniformly distributed throughout the year, indicating a directional tendency to be bi or multivoltine. This was confirmed by Rao's spacing test, which showed significant clustering of brood activity (*X. frontalis*:  $T=313.55$ ,  $p<0.001$ ,  $sd_{circ} = 1.25$ ; *X.*

*grisescens*  $t=295.522$ ,  $p<0.001$ ,  $sd_{circ}=0.36$ ). The circular standard deviation ( $sd_{circ}$ ) of *X. frontalis* indicates a more diffuse distribution.

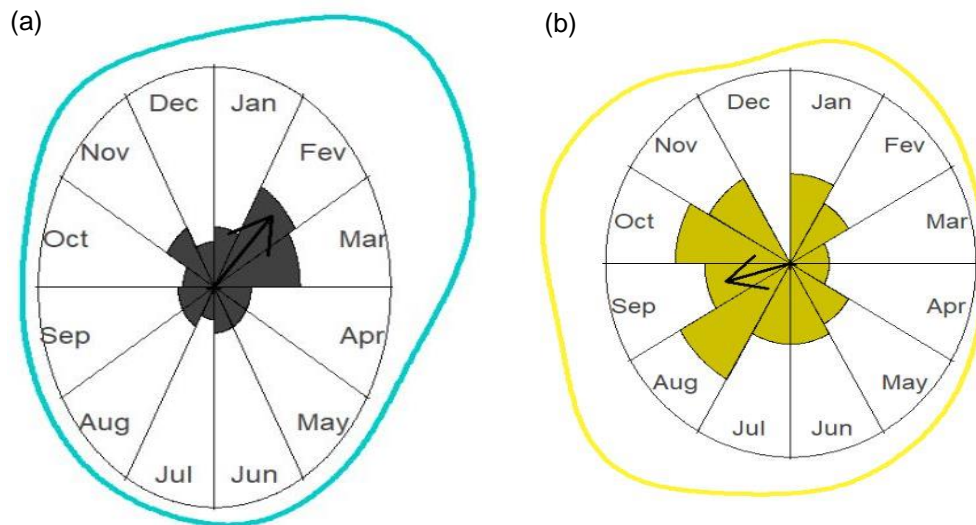
Analyzing the peaks variations throughout the year, we found that *X. frontalis* has its highest peak of cell production in February, representing 60% of the data clustering, with a deviation of  $22.53^\circ$ . The second peak of the species occurs in July with 25% of the data in a much larger dispersion of  $68.46^\circ$ , and the third in November, with 13% of the sample and  $17.32^\circ$  of deviation. *X. grisescens* exhibited its highest data peak around July, accounting for 62% of the data with a standard deviation of  $17.74^\circ$ . The second data peak was observed on October, corresponding to 22% of the samples, with a standard deviation of  $15.98^\circ$ . The third peak was observed on January, corresponding to 16% of the sample, with a standard deviation of  $52.44^\circ$ . The latter peak had a more dispersed cell production in time than the previous peaks.

Finally, after analyzing and describing the data, we performed the Watson-Wheeler test and found that the means of the distributions of cells production by *X. frontalis* and *X. grisescens* are not equal ( $W=35.506$ ,  $p=1.95e-08$ ). Despite the wide distribution and cell production throughout the year, the highest data aggregation occurs in opposite seasons between the species, further demonstrating temporal segregation and seasonal partitioning of reproductive activities.

**Table 2.** Characterization of the brood cells production phenology patterns in *X. frontalis* and *X. grisescens* in Brazilian savanna.

Brood cells production	Length (Vetor r)	Median angle	Rao's Spacing Test
<i>X. frontalis</i>	0.361	$45^\circ$ (Fev. 14)	$313.55 \pm 1.25$ **
<i>X. grisescens</i>	0.457	$255^\circ$ (Sep. 12)	$295.52 \pm 0.36$ **

The significant level of Rao's spacing test: \*\*  $p \leq 0.001$



**Figure 5.** Rose diagrams showing the brood cells production of (a) *Xylocopa frontalis* (b) *Xylocopa grisescens* throughout a cyclic period of a year. Black arrows indicate the median peak for the specie. Blue circle represents the pattern of the fluctuation of brood cells production for *X. frontalis* and yellow circle, for *X. grisescens*.

#### 4. Discussion

We investigated the nesting biology and phenology patterns in brood cells production of *Xylocopa frontalis* and *X. grisescens*. *Xylocopa grisescens* presented longer-lasting nests and more reuse of the bamboo trap-nests while *X. frontalis* exhibited shorter nests cycles and lower reuse. The annual brood cell activity of both species was not uniformly distributed throughout the year. *Xylocopa frontalis* produced 73% of its offspring in the rainy season, while *X. grisescens* produced 60% of its offspring in the middle of the dry season. We observed that both species exhibited a tendency to be bi or multivoltine, and the peak of brood cell production between the species, according to seasonality, can indicate a temporal segregation and partitioning of reproductive activities.

In approximately half of the occupied trap-nests, there was no brood cell production. This behavior was similar in both species and may be a strategy of non-philopatric females, which leave the maternal nest for a new trap-nest, as there are many available substrates, and wait for the best time to disperse from the shelter where they were born. The survival strategy of these species is, to some

extent, guided by constant exploration of new nesting sites, which may favor population survival by maximizing the possibilities of obtaining food resources (Pedroso et al. 2021).

*Xylocopa frontalis* nests exhibit a pattern of fewer consecutive reproductive cycles and less reuse, which suggests a common and dynamic behavior of nest foundation, brood cell production, and high dispersal rates, as also suggested by Pedroso et al. (2021). On the other hand, the observation of longer nests and more individuals remaining in the natal nest, even after their complete physiological development, were common for *X. grisescens* in Brazilian savanna. It also presents higher reuse rates of nests already populated by *Xylocopa* bees, which denotes the social nature of most of these nests in the bee shelters studied (Camillo and Garófalo 1982, Camillo and Garofalo 1989, Chaves-alves 2009).

Despite the production of cells throughout almost the entire year, the annual brood activity for both species was not uniform and the phenology brood cell production for them present different patterns. Both species exhibited a moderate spatial tendency of aggregation in the production of cells, without intense clustering, characterizing both species as bi or multivoltine. However, *X. frontalis* showed a more dispersed distribution of brood cell throughout the year.

*Xylocopa frontalis* exhibited at least two peaks of high brood cell production, with the majority of its reproductive activity during the rainy season, which accounted for 73% of the annual sampling. A small peak was observed in November, while 60% of the annual production occurred in February, at the beginning and end of the rainy season, respectively. We also observed, through the small angular deviation in these peaks, a marked concentration of brood cell production, highlighting the effectiveness in provisioning, which occurred around last than 30 days in both cases. During the dry season, the brood cells production was lower, representing 25% of the sampling, and more dispersed around the peak, with an angular deviation corresponding to more than two months, suggesting a more irregular provision over dry season, which may be related to resource limitation or some type of behavioral control in these populations during this period. These findings are consistent with previous studies conducted in the Brazilian savanna, where two reproductive periods were observed for *Xylocopa frontalis* in the region of Ribeirão Preto, São Paulo state (Camillo and Garofalo

1989). The first occurred from January to March during the rainy season, and the second from July to September during the dry season.

*Xylocopa grisescens* also showed at least two peaks of high brood cell production, with the majority of its reproductive activity during the dry season. Around 84% of the annual sampling occurred around two peaks, the biggest in July (62%) and another in October (22%), but it also presented a smaller peak in the beginning of the year, around January. Camillo and Garófalo (1989) also observed a similar distribution, with a higher brood cells production between December and January and another between August and September.

As *Xylocopa frontalis* is widely distributed in tropical and subtropical regions of the Neotropics, from central Mexico to northern Argentina (Camillo and Garófalo 1982; Moure 2012), it is possible to access its reproductive peaks in other regions of Brazil. In the Atlantic Forest, state of Rio de Janeiro, the reproductive period occurs during the rainy season, between December and April, without the authors characterizing the voltinism of the species (Bernardino and Gaglianone 2013). In Paraná state (Marchi and Melo 2010), also in the Atlantic Forest but much southern, in a subtropical region of Brazil, based on foraging and nest activity analyses, the species was characterized as univoltine. Moreover, Toledo and collaborators (2017) observed a potential for high gene flow for this highly plastic species in Brazilian savanna. This dynamic behavior could potentially enhance the establishment of new nesting sites, favoring population survival and distribution, and reducing inbreeding (Young and Clarke, 2000).

Alternatively, *Xylocopa grisescens* is endemic to the Neotropics and restricted to the Brazilian semiarid and savanna regions, which are xeric habitats (Brasil 2021, Moure, 2012). In the Brazilian semiarid, *X. grisescens* appears to be the most common *Xylocopa* bee, with a higher density in passion fruit and a larger number of nests than *X. frontalis* (Martins et al. 2014). The species presents good gene flow and mobility in Brazilian semiarid regions and seems to have a good ability for tracking resources and crossing anthropogenic barriers, such as agriculture and pasture (Brasil 2021). Further studies are needed to understand the complex nest interactions and adaptability of *X. grisescens* in the Brazilian savanna, a region bordering the species' distribution.

By analyzing the peaks and variation of the angle deviation, it was possible to verify adaptive differences between *X. frontalis* and *X. grisescens* for cell provisioning, which is probably related to their preferences for collecting resources. Araujo et al. (2021) observed that temporal and spatial overlapping species separate their trophic niches to some extent. The main difference found between the pollen collected by *X. frontalis* and *X. grisescens* was the type of anther used, with *X. frontalis* mainly using poricidal pollen, while *X. grisescens* used non-poricidal pollen. The distinguished use of pollen sources by both species observed by Araujo et al. (2021) may be related to the availability and distribution of the plant species that produce these types of pollen. These separation of trophic niches by overlapping species may be a strategy to reduce competition for resources in their environment.

The ability of a habitat to support assemblages of species at different times of the year depends on both seasonality (occurrence of events at specific periods of the year) and predictability (the reliability of event recurrence) of characteristic ecological conditions (Tonkin et al, 2017). Seasonality can explain general phenomena such as life history adaptations, latitudinal diversity gradients, and community structure (McNamara and Houston, 2008).

A seasonal environment with high heterogeneity as the Brazilian savanna, provide a mosaic of microhabitats, such as forest and savanna patches which favors the survival of species of pollinators, especially generalist ones (Oliveira and Gibbs 2000), as *X. frontalis* and *X. grisescens* (Araújo et al. 2021). They often schedule activities regularly throughout the year, and these annual routines could be related to natural selection (McNamara and Houston, 2008). Moreover, the timing of seasonal events, such as flowering and reproduction, is closely linked to environmental conditions and can influence the composition and abundance of species in a given region (Tonkin et al., 2017).

In conclusion, we observed that, despite being sympatric species, *X. frontalis* and *X. grisescens* exhibit different nesting behaviors. Both species have a wide distribution of cell production throughout the year, but the highest aggregation for brood cell production occurs in opposite seasons, showing that the timing of brood activity between the species is correlated to seasonality and influenced by environmental factors. This further demonstrates temporal segregation and some



seasonal partitioning of reproductive activities, facilitating cohabitation in the same bee shelters. However, it also requires different management strategies for the availability of food resources at the appropriate times and maintenance of both populations in new environments in the Brazilian savanna.

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## CAPÍTULO 3

**Colonization of small bee shelters by *Xylocopa frontalis* Oliver in remnants of  
Brazilian Savanna and passion fruit orchard**

## Resumo

*Xylocopa frontalis*, também conhecida como abelha carpinteira, é uma espécie de grande porte e amplamente distribuída na região Neotropical. Nesta espécie, a fundação do ninho é solitária. Contudo, associações entre mãe e filha(s), entre irmãs ou até entre fêmeas não aparentadas podem ocorrer durante os processos de reuso do ninho e, nessas situações, é estabelecida uma divisão reprodutiva de trabalho entre as fêmeas associadas. O comportamento de reuso do ninho, associado ao comportamento filopátricos das fêmeas emergentes, pode proporcionar o incremento de populações dessa espécie por meio da disponibilização de ninhos-armadilha juntamente com um ou mais ninhos ativos em áreas pré-estabelecidas, como cultivos comerciais de maracujá-amarelo, cuja polinização natural depende da presença dessas abelhas. Neste estudo, o objetivo foi avançar na compreensão do processo de colonização de ninho-armadilha (NA) por fêmeas nidificantes de *Xylocopa frontalis*, em pequenos abrigos de abelhas instalados tanto em remanescentes naturais de Cerrado, quanto em cultivos de maracujazeiro. Foram comparadas as taxas de colonização dos NA nessas áreas considerando duas situações: (i) em abrigos de abelhas contendo apenas ninhos-armadilha e (ii) após a introdução de um ninho ativo. Espera-se uma maior taxa de colonização em áreas naturais, menos impactadas por ações antrópicas e na situação (ii) também, devido aos comportamentos filopátricos realizados pelas fêmeas emergentes. Essa abordagem foi eficiente para atrair novas fêmeas, especialmente em áreas naturais. Observamos 27 novas fêmeas e 54 células de cria produzidas em 7 abrigos de abelhas. As populações mais longas e as fêmeas filopátricas foram observadas apenas em áreas naturais, assim como a maioria dos inimigos naturais. Durante a estação chuvosa, foram observados múltiplos ninhos ativos por abrigo, com maior produção de células de cria e um ninho social. No entanto, durante a estação seca, foram observadas restrições reprodutivas associadas a variações comportamentais. Assim, pode-se concluir que o uso de pequenos abrigos de abelhas com ninhos ativos é um método viável para iniciar e cultivar populações de *Xylocopa frontalis* em áreas pré-estabelecidas por longo prazo.

**Palavras-chave:** Abelha carpinteira, mamangava de toco, abrigo de abelhas, novas colonizações, inimigos naturais, cerrado.

## Abstract

*Xylocopa frontalis*, also known as the carpenter bee, is a large species widely distributed in the neotropical region. In this species, the nest foundation is solitary. However, associations between mother and daughter(s), between sisters, or even between unrelated females can occur during the nest reuse processes, and in these situations, a reproductive division of labor is established between the associated females. The nest reuse behavior, associated with the philopatric behavior of emerging females, can provide an increase in populations of this species, through the availability of trap-nests, along with one or more nucleating nests in pre-established areas, such as commercial yellow passion fruit crops, whose natural pollination depends on the presence of these bees. In this study, the objective was to advance in the understanding of the nest colonization process of *Xylocopa frontalis* nesting females in trap-nests (TN) in small bee shelters installed both in natural remnants of the Brazilian savanna and in passion fruit crops. The rates of TN colonization in these areas were compared considering two situations: (i) in bee shelters containing only trap-nests and (ii) after the introduction of an nucleating nest. Our expectation was that the colonization rate would be higher in natural areas, less impacted by anthropic actions, and in situation (ii) as well, due to the philopatric behaviors performed by emerging females. This approach was efficient in attracting new females, especially in natural areas. We observed 27 new females and 54 brood cells produced in 7 bee shelters. Longer populations and philopatric females were observed only in natural areas, as well as most of the natural enemies. During the rainy season, multiple active nests per shelter were observed, with greater brood cell production and a social nest. However, during the dry season, restrictions on brood cell production were observed. In conclusion, the use of small bee shelters with nucleating nest is a method viable to start and grow *Xylocopa frontalis* populations in pre-established area for long-term.

**Keywords:** Carpenter bees, bee shelters, natural enemies, new colonization, Brazilian savanna

## 1. Introduction

*Xylocopa frontalis* (Olivier, 1789), also known as the giant carpenter bee, may be the most widespread carpenter bee in the Neotropics region, occurring from central Mexico to northern Argentina, and is very abundant in several ecosystems in Brazil (Camillo and Garófalo 1982; Moure 2012). The females of this species build their nests excavating in dead wood, dry or rotten branches (Michener 1974, 2007; Hurd 1978; Roubik 1989), and also nest in bamboo trap-nests (Chaves-Alves *et al.*, 2011). The species females remain totipotent throughout adulthood and are able to found nests of their own (Camillo and Garófalo, 1989). The choice of substrates for nesting is made solitarily, where after excavating the nest channel, females begin construction and provisioning of brood cells (Roubik, 1989).

In this populations, nests can range from solitary to social colonies with highly variable organization. Studies have shown interactions between conspecifics, including feeding behavior (trophallaxis) (Gerling *et al.* 1989) and the reactivation of old nests by new females (Camillo and Garófalo, 1989, Chaves-Alves *et al.*, 2011, Pedroso *et al.*, 2021). Interactions between females in the construction, provisioning, and guarding of nests, oviposition, and competition for substrates for nesting have also been observed in different species (Velthuis and Gerling, 1983; Camillo e Garófalo, 1989, Hogendoorn and Leys, 1993; Hogendoorn and Velthuis, 1993 and 1995; Hogendoorn, 1996). This nest joining behavior has been observed in several *Xylocopa* species and may create opportunities for association, including among non-relatives (Gerling, 1982; Velthuis, 1987; Camillo and Garófalo, 1989; Peso and Richards, 2011).

After provisioning the brood cells, during the development of the immatures, the nesting female of *Xylocopa* species remains in the nest, guarding her young, and only rarely goes out to forage (Buchman and Minckley, 2019). This behavior is probably related to the many natural enemies known to attack large carpenter bees, such as ants, flies, blister beetles and parasitoid wasps (Watmough, 1983, Rocha-Filho *et al.* 2021), and of protecting brood from pollen robbery of conspecifics (Dunn and Richards, 2003).

Moreover, *Xylocopa* bee's species presents philopatry behavior, a tendency to reproduce at the natal place, that is stronger in nest-specialist bees (Lopez-Uribe et al., 2015), such as the studied carpenter bee *X. frontalis* and *X. virginica* (Camillo and Garófalo, 1989, Chaves-Alves et al., 2011, Ballare e Jha, 2021). They are also long-distance dispersers, buzz-pollinators, and generalists regarding the collection of food resources (Araújo et al. 2021).

As illustrated above, *Xylocopa* bees are an excellent model to study artificial shelter colonization, nesting behavior, and reproductive cycles since both dispersive and philopatric behaviors can be observed and may lead to complex social behaviors, once their longevity open the opportunity for repeated social interactions across different generations (Buchman and Minckley, 2019).

Studies on the nest biology of those bees using trap-nests have provided valuable information for their conservation in natural environments as well as for their management in agroecosystems (Freitas and Oliveira Filho, 2001; Pereira, 2002; Oliveira Filho e Freitas 2003, Junqueira e Augusto, 2017).

Junqueira et al. (2012) observed in short-time experiments that supplying a bee shelter with a combination of suitably sized empty bamboo stalks and active nests of carpenter bees can enhance the efficiency of management techniques to minimize pollination deficit in passion fruit crops, as a consequence of the emergence of brood from the introduced nests and the attraction of bees from the surroundings.

In this study, the objective was to advance in the understanding of nest colonization process of *Xylocopa frontalis* nesting females in trap-nests (TN) in small bee shelters installed both in natural remnants of the Brazilian savanna and in passion fruit crops. The rates of TN colonization in these areas were compared considering two situations: (i) in bee shelters containing only trap-nests and (ii) after the introduction of an nucleating nest. Our expectation was that the colonization rate would be higher in natural areas, less impacted by anthropic actions, and in situation (ii) as well, due to the philopatric behaviors performed by emerging females.



## 2. Methods

### 2.1 Artificial small bee shelter's

Trap-nests are artificially constructed nesting boxes that mimic the natural nesting resources of cavity-nesting bees and wasps and are used for the study and management of those bees.

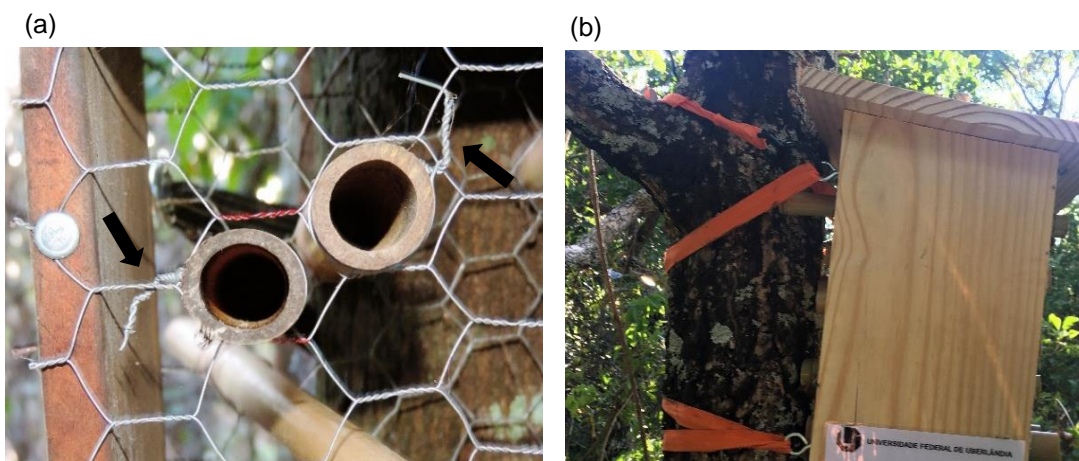
We constructed small, square wooden bee shelters measuring 30cm in width, 40cm in height, and 15cm in depth for easier handling and installation to initiate new populations of *Xylocopa frontalis* in the studied areas. The roof was wider at 25cm in front, inclined, to prevent the entry of nests in the occurrence of windy rains and with a handle for easy transportation. To prevent the bee shelter's wood from absorbing moisture and developing mold, the finished structure was burned with oil using a portable blowtorch.

We closed the wooden box by screwing a galvanized hexagonal wire screen with a mesh size of 1 inch to the front and back. This mesh size is ideal for introducing nests with diameters between 1.4cm and 2.4cm, suitable for *Xylocopa* bees. We also attached 50mm PVC pipes underneath the bee shelter to provide trap-nests with smaller length and diameter, intended for other groups of smaller bees. These additional trap-nests were designed to accommodate various cavity-nesting species (Figure 1).

The trap-nests, consisting of bamboo segments sawed in a way that one side remained closed by the bamboo node and the other end open. It is important to note that the trap-nests needed to have a size larger than the minimum depth of the bee shelter (15cm) to ensure proper nesting attachment. Furthermore, to provide greater stability and security to the bamboo trap-nests, we twisted rings with malleable aluminum wires on each trap-nest and locked them onto the grid (Figure 2a). We inserted four closed hooks at the back of the bee shelter for attachment to trees with a ratchet (Figure 2b), at approximately 1.5m from the ground. We also used entomological glue and tape to prevent the occupation of the trap-nests by ants, spiders, crickets, and other arthropods, when possible.



**Figure 1.** Small bee shelters installed in all study areas.



**Figure 2.** (a) Trap-nests properly positioned for occupation by *Xylocopa* bees. The arrows highlight the twisted rings holding the bamboo and providing stability for intranidal behaviors of the females, such as excavation. (b) Bee shelter installed at the ideal height, placed in position by the hooks and tighten with a ratchet strap.

## 2.2 Study areas

We installed these small bee shelters in 20 areas, nine in remnants of Brazilian savanna (cerrado sensu stricto) and eleven on the side of passion fruit orchards in the Triângulo Mineiro region, in the municipalities of Araguari, Uberlândia, and Indianópolis, Minas Gerais state, Brazil (Table 1). We provided 20 trap-nests with diameter between 1.4cm and 2.4cm in the hexagonal wire screens of each small bee shelter.

Due to episodes of fire that destroyed the bee shelters, one natural area (CER09) and one passion fruit area (MCJ01) had their observations interrupted in the dry season of 2020. MCJ04 and MCJ06 was also interrupted later in the experiment.

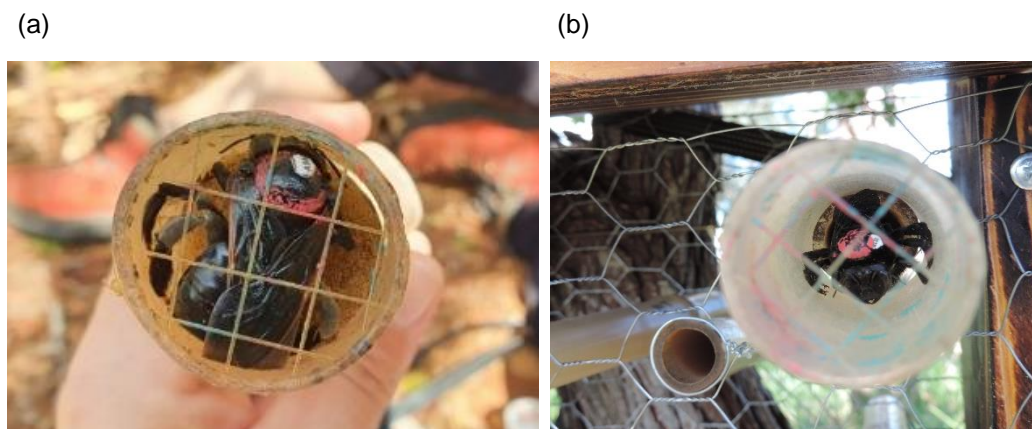
## 2.3 Introduction of *Xylocopa frontalis* nests and monitoring of bee shelters

To initiate the colonization in the shelters and attract other nesting females present in the areas, it was made the introduction of one *Xylocopa frontalis* nests were made in seven of nine bee shelters installed in remnants of Brazilian savanna (CER01, CER02, CER03, CER04, CER05 e CER06 and CER09) and five of eleven (MCJ01, MCJ2, MJC3, MCJ5 and MCJC11) in passion fruit orchards (Table 1). The introduction was made in two different moments: *Early*, from 0 to 45 days after the installation of bee shelter (CER01, CER02, CER03, CER06, CER09, MCJ01, MCJ2 and MCJ5) and *Later*, from seven to nine months (CER04, CER05, MJC3, MCJC11). In the remaining areas, the bee shelters installed were maintained with only trap-nest (CR07, CR08, MCJ07, MCJ08, MCJ09 and MCJ10) (Table 1). Two area had the monitoring interrupted (MJC4 and MJC6) due the conditions of crops.

The nests introduced were collected from breeding areas at the Panga Ecological Station (PES) and Experimental Station of Água Limpa (ESAL), both from Federal University of Uberlândia (UFU).

Monthly observations of the trap-nests were conducted, from January 2020 to September 2022. During the visits to study area, we collected information about the number of active nests, the number of cells produced and nest behaviors.

To allow for individual tracking of the females present in the active nests, each one was labeled with a numerical tag attached to its mesothorax (see YAMAMOTO, 2009), and markings with different colored ink were used to differentiate generations and facilitate behavioral observations (Figure 3a-b).



**Figure 3.** Handling and marking *X. frontalis* bees in small bee shelters according to Yamamoto's (2009) methodology.

#### 2.4 Data analysis

We used Generalized Linear Models (GLM), with R-package *glmmTMB* version 1.0.2.1 (Brooks et al., 2017), to test if new nest foundations were different between areas classification (natural areas and crop areas). As the data had zero inflated distribution, we used Negative binomial distribution family (nbinom2) (Hardin & Hilbe 2007).

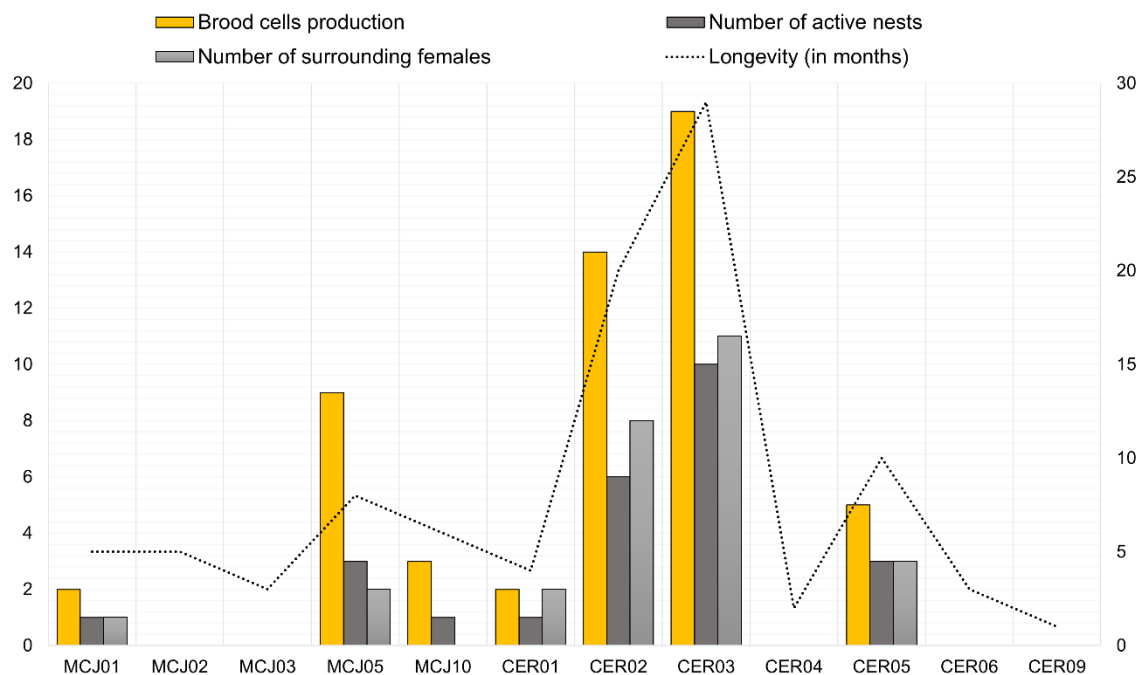
To test for differences in brood cells production between seasons, we used Linear Models (LM).

**Table 1.** Selected study areas in the region of Araguari, Uberlândia and Indianópolis, Minas Gerais.

<b>Small bee shelter areas</b>	<b>Code</b>	<b>Classification of areas</b>	<b>Coordinate</b>	<b>Shelter installation</b>	<b>Introduction of nucleating nests</b>
Panga Ecological Station	CER01	Natural	-19,170616 -48,390646	16/01/2020	16/01/2020
Itororó Hunting and Fishing Club	CER02	Natural	-18,983604 -48,294087	16/01/2020	16/01/2020
Private Reserve Eucatex	CER03	Natural	-19,039168 -48,091393	07/02/2020	07/02/2020
Reserve in cattle farm 1	CER04	Natural	-18,841782 -48,396616	26/02/2020	26/11/2021
Reserve in cattle farm 2	CER05	Natural	-18,907336 -48,529666	26/02/2020	26/11/2021
Reserve in cattle farm 3	CER06	Natural	-19,033085 -48,535143	03/03/2020	01/04/2020
Private Reserve Faber Castell	CER07	Natural	-19,120305 -48,630557	03/03/2020	Not introduced
Reserve in cattle farm 4	CER08	Natural	-19,390757 -48,423420	02/03/2020	Not introduced
Reserve in cattle farm 5	CER09	Natural	-18,960614 -48,164917	04/03/2020	01/04/2020
Farm 1	MCJ01	Agricultural	-18,514561 -48,020113	14/01/2020	17/01/2020
Farm 2	MCJ02	Agricultural	-18,935119 -47,897424	03/03/2020	17/04/2020
Farm 3	MCJ03	Agricultural	-18,875564 -47,979774	03/03/2020	26/11/2021
Farm 4	MCJ04	Agricultural	-18,569224 -48,018170	14/01/2020	Interrupted
Farm 5	MCJ05	Agricultural	-18,808951 -47,976645	04/03/2020	03/04/2020
Farm 6	MCJ06	Agricultural	-18,584725 -48,407499	04/03/2020	Interrupted
Farm 7	MCJ07	Agricultural	-18,552112 -48,172807	04/03/2020	Not introduced
Farm 8	MCJ08	Agricultural	-18,499708 -48,142287	04/03/2020	Not introduced
Farm 9	MCJ09	Agricultural	-18,637544 -48,056216	04/11/2020	Not introduced
Farm 10	MCJ10	Agricultural	-18,475660 -48,264321	16/03/2021	Not introduced
Farm 11	MCJ11	Agricultural	-18,641488 -48,258995	16/03/2021	26/11/2021

### 3. Results:

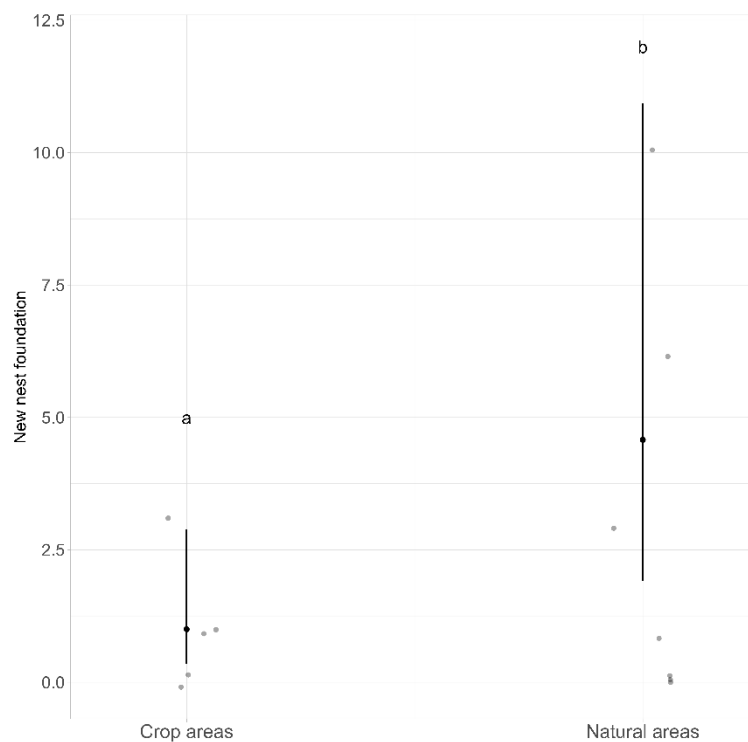
We verified the nests foundation only in areas that occurred the introduction of one nucleating nest of *X. frontalis*. The introduction of small bee shelters followed by the introduction of a nucleating nest of *X. frontalis* resulted in the attraction of 27 new females and the production of 54 brood cells in seven bee shelters, four installed in remnants of Brazilian savanna areas and three in passion fruit orchards (Figure 4, Table 2). The remnants of Brazilian savanna provided better conditions for the development of this population, attracting 88% of the new females from the surrounding areas and 65% of brood cells production. The number of nests founded was significantly higher in remnants of Brazilian savanna compared to passion fruit orchards (Chisq=4.7366, p=0.02953) (Figure 5).



**Figure 4.** Number of brood cells produced, active nests, and surrounding females in all areas with introduction of a nucleating nest. The longevity of bee shelters (in months) shown on the secondary axis.

**Table 2.** Number of trap-nest occupation, nest foundation, brood cells production, surrounding females, and longevity of *Xylocopa frontalis* populations after the introduction of a nucleating nest in bee shelters installed in remnants of Brazilian savanna and passion fruit orchards, Triângulo Mineiro, Minas Gerais State, Brazil.

Small Bee Shelters	Trap-nest occupation	Nest founded	Brood cells production	Number of surrounding females	Longevity (in months)
MCJ01	2	1	2	1	5
MCJ02	1	0	0	0	5
MCJ03	1	0	0	0	3
MCJ05	5	3	9	2	8
MCJ10	1	1	3	0	6
CER01	3	1	2	2	4
CER02	20	6	14	8	20
CER03	25	10	19	11	29
CER04	1	0	0	0	2
CER05	3	3	5	3	10
CER06	1	0	0	0	3
CER09	1	0	0	0	1



**Figure 5.** Number of nests founded in bee shelters installed in remnants of Brazilian savanna and passion fruit orchards, Triângulo Mineiro, Minas Gerais State, Brazil.

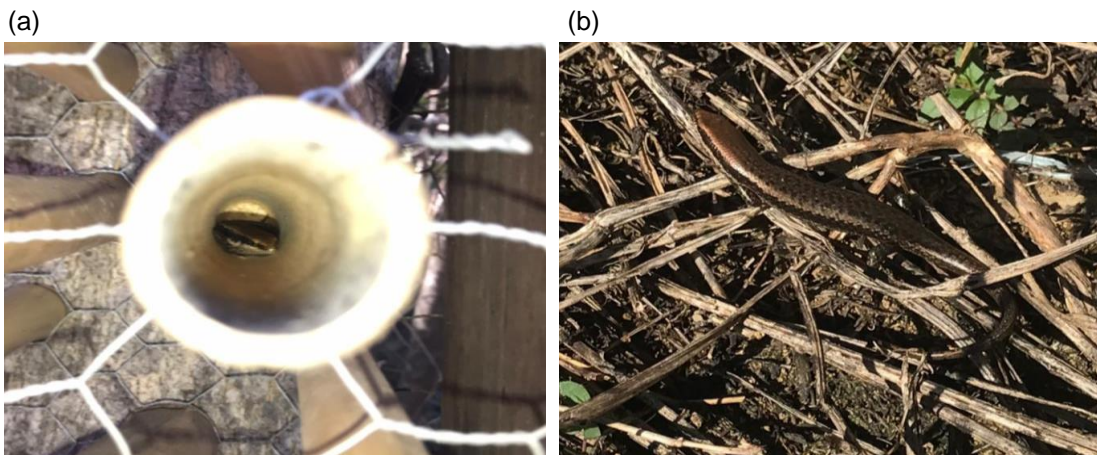
In the crop areas, we observed a longer nesting presence in MCJ5, which remained active for 8 months, mostly with the presence and brood cell production of the introduced female. This female produced 7 brood cells using 2 nests simultaneously and dispersed all the offspring produced during the dry season right after the introduction. We believe that this bee used a flower blooming episode in the surrounding of the shelter to maximize her fitness after the change of habitat. We also observed two new females from the surrounding in MCJ05. The only other crop area that had nesting females from the surrounding was MCJ01, with one new female before the fire event.

### 3.2 Natural enemies

Regarding natural enemies, in 2 out of 3 crops areas where introduction was effective and one of the bees produced brood cells in the shelter, we observed predation by the skink lizard *Notomabuya frenata* Cope (Squamata, Mabuyidae). In all cases, followed by complete dispersal of the female bees from the shelter (Figure 6). In MCJ05, we verified the presence of the predator inside the bee bamboo trap-nest with the shelter already empty of *Xylocopa* bees and a month later, we observed the lizard female incubating there's eggs on top of the PVC pipes. In MCJ01, only the cell was broken in the same pattern observed in MCJ05, with the operculum broken in the lower portion, leaving the larva exposed, when it was still present.

In the remnants of Brazilian savanna, we observed three different natural enemies. In CER02, at the end of February 2021, an abandoned nest with pollen mass and predation of the 1st cell by the ant *Crematogaster* was observed. These tiny ants opened a small access through the sawdust of the opercula and consumed the developing larva. In CER03, we identified the development of *Cissites maculata* larvae (Coleoptera: Meloidae) inside the nest. This species is an obligatory cleptoparasite of *Xylocopa* bees (Marchi and Melo, 2010) and consumed three brood cells (Figure 7). We also observed in CER03 two other nests with three pupae in two nests parasitized by an unidentified natural enemy (Figure 8).

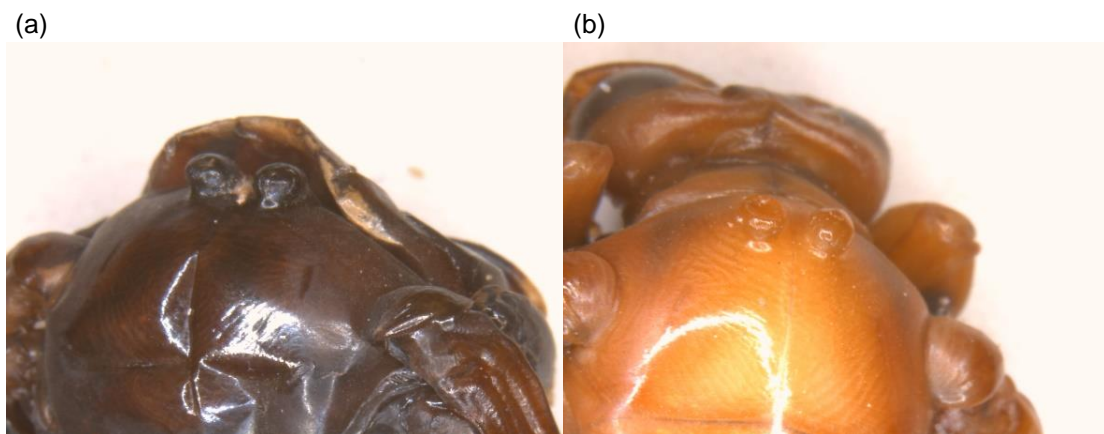




**Figure 6.** Lizard *Notomabuya frenata* (Squamata, Mabuyinae): (a) inside a bamboo trap-nest, (b) in the crop around the small bee shelter in MCJ05.



**Figure 7.** Larva of *Cissites maculata* (Coleoptera: Meloidae) developing inside the trap-nest of *Xylocopa frontalis* in CER03.



**Figure 8.** Pupa parasitized by an unidentified natural enemy in different trap-nets in CER03. (a) female pupa, (b) male pupa.

### *3.2 Behaviors of reuse and occurrence of social nests*

The CER02 and CER03 areas produced together 33 new brood cells in the span of 20 and 29 months. We observed the occurrence of philopatric females, those born in the shelter who remain to reproduce and/or assist other female, in 3 out of 7 natural areas (CER02, CER03, CER05), and none in the crop areas.

In shelters with longer-lived populations such as CER02 and CER03, the females in both bee shelters presented frequent reuse of trap-nests, with 22 occupations of previously used trap-nests (CER02: 9, CER03: 13). In CER02, we also observed the production of a second philopatric generation and a social nest. Moreover, we found in CER02 e CER03 significant differences in effective cell production (the number of cells that are successfully provisioned and result in the emergence of bees) between seasons ( $\text{Chisq}=7.202$ ,  $p=0.0072$ ), with  $4.8\pm 1.01$  cells in the wet season and  $0.75\pm 1.12$  in the dry season.

During the wet season, multiples active nests per shelter were observed, with up to four in CER03 during the wet season of 21/22, in the second year of observations. The number of cells produced was the highest in CER02 during the 20/21 season reaching up to ten cells in the bee shelter, 6 of them in one social nest observed from February to April of 2021. In all wet seasons monitored we observed the continuous production of brood cells in nests.

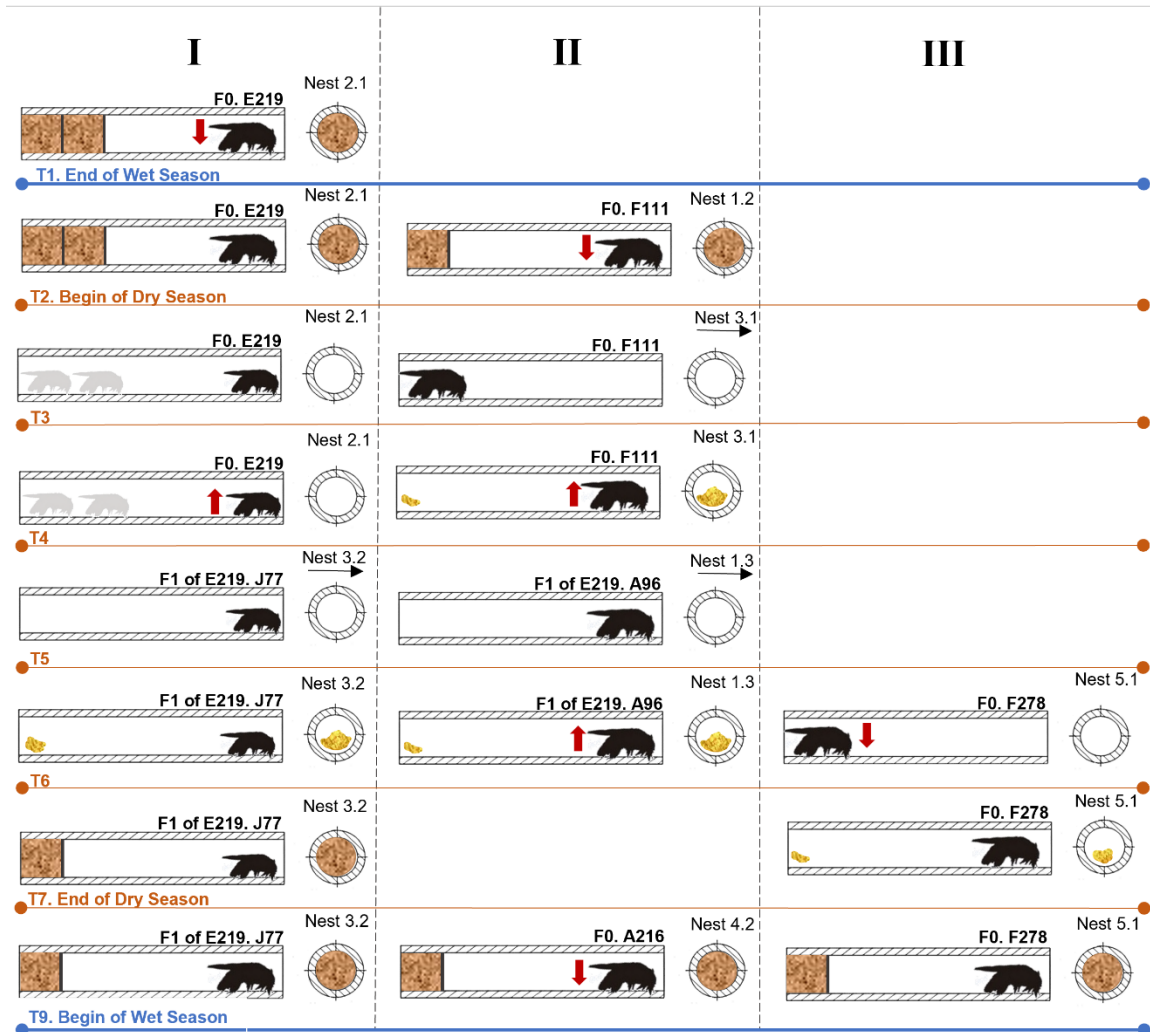
In February, during the species' reproductive peak, we observed a philopatric female in another nest with a new adult female, with less wing wear than the philopatric female and for that, probably younger. We marked the new female and recorded the presence of three brood cells finished in the social nest, with the philopatric female observed exhibiting behavior consistent with a beta or subordinate female, scraping the bamboo for the formation of the next offspring cell and subsequent guarding at the nest entrance. The new female in this social nest, the alpha or dominant female, was the one that performed the foraging trips, collecting pollen and nectar, and probably also performing the oviposition in all or most of the brood cells production. The social nest was the one that presented the highest number of cells in a single reproductive cycle in all observations, reaching

the limit of the substrate size, maintaining only a small vestibule and six brood cells, with the dispersion of the alpha female after the conclusion of brood cells production.

In the dry season, we recorded the beginning of the production of up to three consecutive nests by a single bee in both bee shelters. However, effective offspring production was observed in only one of the occupied nests in each bee shelter (Figure 9: CER02-I: T6; Figure 10: CER03-III: T5), with a registry of not close opercula, in a gap of a week, by the reproductive female (Figure 10: CER03-III: T5 and T6. The usurped females changing the trap-nest after discontinuing there's cells production. In 71% of the cases, these females used reuse nests in the new beginning of cells production (Figure 9: CER02-I: T4 and T6, CER02-II: T4 and T7; Figure 10: CER03-I: T1 and T4, CER03-II: T2 and T4).

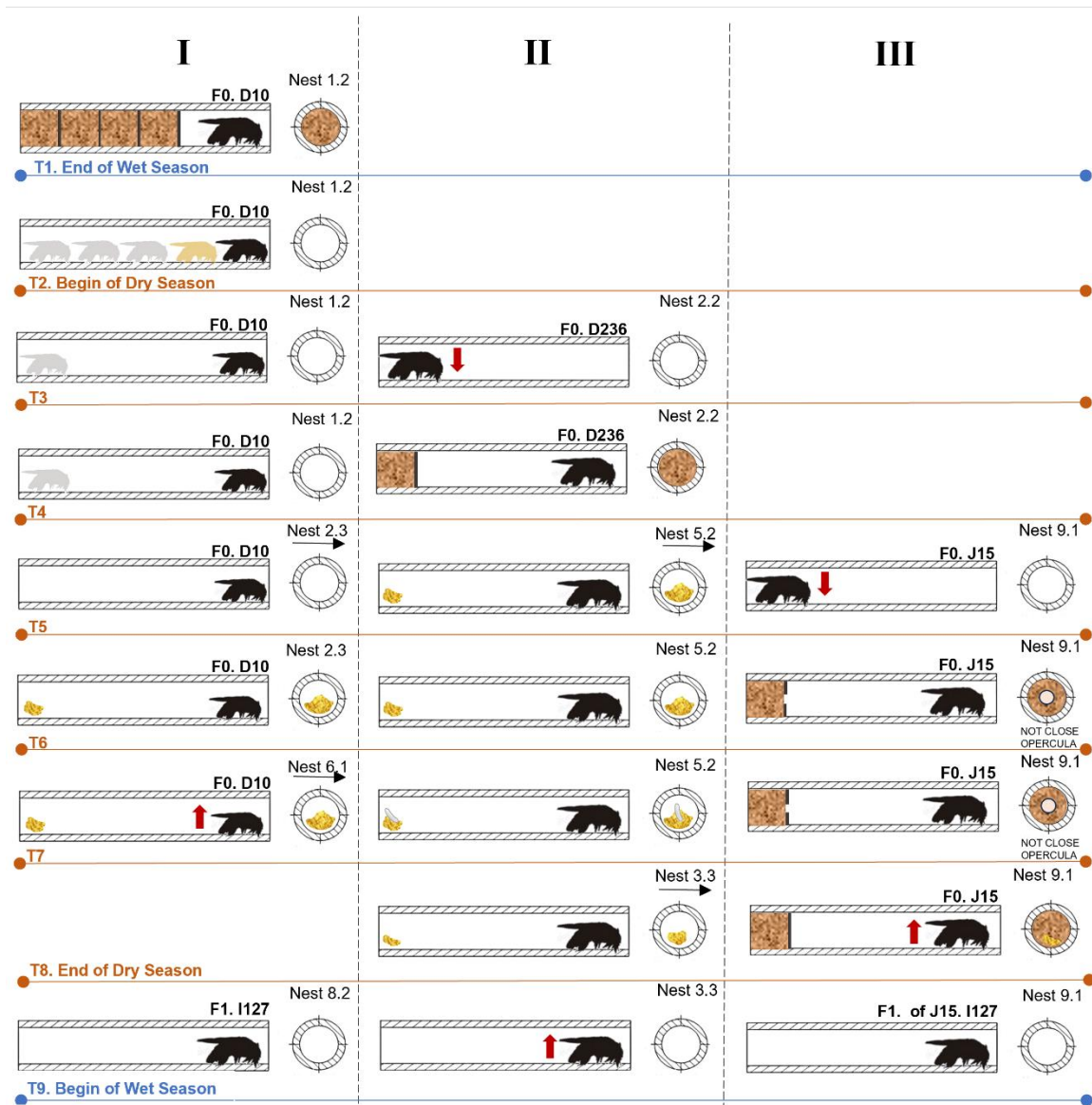
Pollen robbery and cell destruction were observed in different parts of the construction process, including inferred oophagy and the destruction of complete cells in different trap-nests. These behaviors were observed between June and August in CER03 and August and October in CER02. The pollen robbery, contrary to expectations, was not directed towards the production of more than one cell by any of the bees in the shelter, suggesting a territorial behavior in this new bee shelters. At the end of the dry season, females that failed in reproduction dispersed.

## CER 2, 2020



**Figure 9.** Flowchart of nesting behaviors observed in a weekly or bi-weekly monitoring in CER02. In each representation of the nest, on the left side, we observe a lateral view of the nest, and on the right side, we observe a frontal view. The brown pattern symbolizes the brood cells, subdivided by black vertical lines. The horizontal black arrows represent the change of nest by a female, the vertical red arrows represent the arrival and dispersal of females. Light gray represents young females. “F0” is the founding female and “F1” the first generation produced in the bee shelter.

### CER 3, 2021



**Figure 10.** Flowchart of nesting behaviors observed in a weekly or bi-weekly monitoring in CER03. In each representation of the nest, on the left side, we observe a lateral view of the nest, and on the right side, we observe a frontal view. The brown pattern symbolizes the brood cells, subdivided by black vertical lines. The horizontal black arrows represent the change of nest by a female, the vertical red arrows represent the arrival and dispersal of females. Light gray represents young females and light yellow a young male. “F0” is the founding female and “F1” the first generation produced in the bee shelter.

#### 4. Discussion

The introduction of small bee shelters and nucleating nests of *X. frontalis* was efficient to attract new females, especially in natural areas that provide better environmental conditions. We also observed that in areas with only the introduction of unused bamboo trap-nests, without the introduction of a nucleating nest, there was no spontaneous nesting of *Xylocopa* bees.

The decline of pollinators is being exacerbated by the increasing fragmentation and loss of natural habitats, leading to lower availability of substrate and food sources for bees (Freitas and Oliveira-Filho 2003; Pinheiro and Freitas 2010). We observed that *Xylocopa* bees prefer to nest in natural areas in Brazilian Savana (*cerrado sensu stricto* areas) rather than in the sideways of passion fruit crops. Reduced habitat area or low diversity in the surrounding can increase the impact of limiting resources for population growth, which can lead to greater dispersal, as observed in our crop's areas. In contrast, we observed better results of new female nesting in the shelters and higher cell production in natural areas. The extent of dispersal and nest relocation across years may also depend on factors such as population density and the degree of intraspecific competition (Ostwald, 2021).

According to previous study in the same region by Junqueira and Augusto (2017), we confirm that the introduction of a nucleating nest within the bee shelters helps the establishment of new populations in a given area. The bee shelters without the presence of *Xylocopa* bees do not have a spontaneous nesting probable due to the lack of olfactory marks utilized by flying insects for nest recognition (Vandernabeele and Schmitt 2023) that may help in the identification of the bee shelter. The high nest reuse should also be related to this olfactive clues.

The distribution of resources for food and nesting, as well as the presence of natural enemies, are the main factors that can constrain the development of *Xylocopa* bees (Steffan-Dewenter and Shiele, 2008).

The *Mabuyidae* lizards have not been recorded preying on *Xylocopa* bee's larva in their shelters before, suggesting a new resource adaptation of the lizard,

which feeds on insects among other things (Machado, 2022), once these lizards are typically observed in forest formations and not in agricultural areas, as recorded here. The predation of *Mabuyidae* lizards on *Xylocopa* bees is a concerning issue, as they apparently prey all the brood cells in the nests and lead to the dispersion of the adult population. The *Cissites maculate* beetle is a common natural enemy of *Xylocopa* bees (Bernardino and Gaglianone 2013, Pereira and Garófalo 2010) and the predation of pupae by ants was previously observed for the *Camponotus* ant (Marchi and Melo 2010), but not for *Crematogaster*.

The extreme longevity of *Xylocopa* allows the option of delaying reproduction until later in the same season or the following season if current conditions are not appropriate and this usually leads to complex interactions between females, because there is also an influence of intraspecific competition for reproductive dominance and philopatric behavior in those bees (Pedroso et al., 2021).

Climatic factors affect the decision between staying or dispersing, defining the population dynamics in several *Xylocopa* species (Hogendoorn and Leys, 1993) and increasing environmental constraints lead to higher reproductive skew in social nests (Hogendoorn and Velthuis, 1999), and can also drive cyclical variation in social behavior, as temporary grouping behavior can maximize seasonal opportunities for resource exploitation (Bos et al., 2004).

In this newly established population of bees in natural areas, we observed strong signs of competition for single reproduction in the bee shelter during the dry season, suggesting seasonal variations in behavior. We observed pollen robbery and the reproduction of a single nest with a single female at the end of the dry season. This aggressive behavior may be related to the new colonization of the bee shelter nesting sites and to the high availability of free nests, which is not common for these bees in natural wood nest aggregations.

In contrast to the greater tolerance observed for females during winters (Gerling et al., 1989; Hogendoorn and Velthuis, 1993; Ostwald et al., 2020), we observed greater tolerance between different nests during the rainy season for *Xylocopa frontalis*, a plastic and widely distributed neotropical species. During the rainy season, there was a higher density of brood cells, which may have led to

greater competition within the population during the dry season, when resources were more restricted.

The social nest observed presented the highest production of cells in a single nest throughout the experiment, with six brood cells, which denotes an increase in fitness in these social nests (Reyer, 1984; Ostwald, 2020). In this case, the beta female was a philopatric female of the shelter, which may be related to the alpha female that arrives and takes dominance of the nest. To better understand these genetic relationships between the species, further analyses of kinship are necessary.

Considering the complex behaviors in the nests, our study suggests that allocating more than one small bee shelter within native forests could be a more appropriate management strategy for *Xylocopa* bees. These areas provide conditions of greater climate regularity and ecological integrity, favoring the provision of necessary resources for survival (Pedroso, 2021). Moreover, to start and grow *Xylocopa frontalis* populations, we propose introducing a nest with a female and brood production, according to the best outcome observed by Junqueira and Augusto (2017) in management experiments. These bees are highly dynamic, exhibit philopatric interactions and complex behaviors that lead to seasonal variation in social behavior. These behaviors can lead to complex sociality, especially in bamboo trap-nests, where the bees do not have the expensive energetically cost of nest construction (Peso and Richards, 2011; Ostwald et al., 2021), ultimately improving their fitness and the size of this populations in the medium to long term.

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## CONSIDERAÇÕES FINAIS

Os capítulos apresentados neste trabalho discutem informações relevantes sobre a polinização do maracujá amarelo e a dinâmica populacional de duas espécies de abelhas *Xylocopa* frequentemente observadas em ninhos-armadilha no Cerrado, *Xylocopa frontalis* e *Xylocopa grisescens*.

No Capítulo 1, verificamos que os cultivos de maracujá são altamente dependentes de abelhas de grande porte e, na região estudada, *X. frontalis* foi o único polinizador efetivo do cultivo. A produtividade do maracujá é negativamente afetada pela presença de pilhadoras exóticas, representados exclusivamente pelas abelhas *Apis mellifera*. Por outro lado, as abelhas do gênero *Trigona* também são pilhadoras, mas não apresentaram efeito negativo na produtividade do maracujá. Tanto a produção quanto a quantidade de polpa produzida no maracujá, independentemente da polinização manual ou natural, estão relacionadas a déficits de polinização nas áreas, enquanto a doçura da polpa é maior nos tratamentos de polinização natural. A maior produtividade do maracujá-amarelo e a abundância das duas principais espécies de abelhas (*X. frontalis* e *A. mellifera*) que influenciam esta produtividade estão relacionados ao entorno dos cultivos de maracujá. Áreas com uso moderado do solo, como pastagens, estão positivamente relacionadas a maior produtividade, enquanto áreas com intenso uso do solo, como culturas de soja, influenciam negativamente esta produção. Além disso, a cobertura vegetal está positivamente correlacionada com a abundância de *Xylocopa frontalis* e negativamente correlacionada com a abundância de *Apis mellifera*. Já o intenso uso do solo apresentou o comportamento oposto, sendo positivamente relacionado a abundância de *Apis mellifera* e negativamente relacionado a abundância de *Xylocopa frontalis*.

No Capítulo 2, observamos que *Xylocopa frontalis* e *Xylocopa grisescens*, apesar de serem espécies simpátricas, apresentam comportamentos de nidificação diferentes, com *X. grisescens* apresentando ninhos mais longos e mais povoados. Ambas as espécies têm uma ampla distribuição de produção de células ao longo do ano no cerrado e se mostraram bi- ou multivoltinas. A maior agregação de produção de células de cria ocorre em estações opostas, com *X. frontalis* apresentando maior produção de células de cria na estação chuvosa e *X.*

*grisescens* na estação seca, mostrando que o período de nidificação entre as espécies está correlacionado com a sazonalidade e influenciado por fatores ambientais. Isso demonstra alguma segregação temporal e partição sazonal das atividades reprodutivas, facilitando a coabitação das espécies nos mesmos abrigos de abelhas.

No Capítulo 3, observamos que nas áreas naturais de Cerrado houve uma maior atração de novas fêmeas para os abrigos. Também foi observado um maior número de células de cria e maior tolerância entre as fêmeas de diferentes ninhos durante a estação chuvosa. Observamos roubo de pólen e sinais de competição por reprodução nos abrigos de abelhas durante a estação seca. O menor custo desses ninhos-armadilha de bambu pode facilitar a manutenção de mais de um abrigos de abelhas na mesma área, visando evitar as interações negativas entre as fêmeas nidificantes e promover o aumento das populações de *X. frontalis* a médio e longo prazo.

Em conclusão, considerando o manejo de polinizadores e a produtividade dos cultivos de maracujá, sugerimos: (i) delinear o plantio de cultivos e estratégias de manejo longe de áreas de uso intensivo do solo e próximas a áreas naturais como uma prática amigável às abelhas, que pode favorecer o rendimento do maracujá e ajudar a manter a presença e densidade de *Xylocopa* na região e, por consequência, nos cultivos de maracujá; (ii) estabelecer estratégias de manejo para a disponibilidade de recursos alimentares concentrados nas preferências reprodutivas de *X. frontalis* na estação chuvosa e de *X. grisescens* na estação seca para a manutenção de ambas as populações em novos ambientes no cerrado; (iii) introduzir dois ou mais abrigos de abelhas pequenos em áreas pré-estabelecidas, com pelo menos um deles com um ninho nucleador para manter essas populações em desenvolvimento e prover a área com ninhos-armadilha em uma estratégias de médio a longo prazo.