UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE

ISABELLE CRISTINA SANTOS MAGALHÃES

POLINIZAÇÃO E SUA INFLUÊNCIA NA PRODUÇÃO DE CULTURAS DE CUCURBITACEAE

RECIFE – PE
2024

ISABELLE CRISTINA SANTOS MAGALHÃES

POLINIZAÇÃO E SUA INFLUÊNCIA NA PRODUÇÃO DE CULTURAS DE CUCURBITACEAE

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biodiversidade da Universidade Federal Rural de Pernambuco como requisito parcial para a obtenção do grau de Doutora em Biodiversidade.

Orientadora: Profa. Dra. Cibele Cardoso de Castro

RECIFE – PE

2024

Dados Internacionais de Catalogação na Publicação
Sistema Integrado de Bibliotecas da UFRPE
Bibliotecário(a): Auxiliadora Cunha – CRB-4 1134

M189p Magalhães, Isabelle Cristina Santos.
Polinização e sua Influência na Produção de Culturas de *Cucurbitaceae* / Isabelle Cristina Santos Magalhães. – Recife, 2024.
118 f.; il.

Orientador(a): Cibele Cardoso de Castro.

Tese (Doutorado) – Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Biodiversidade, Recife, BR-PE, 2024.

Inclui referências e apêndice(s).

1. Hortaliças. 2. Polinizadores. 3. Abelhas. 4. Polinização por insetos 5. *Cucurbita*. I. Castro, Cibele Cardoso de, orient. II. Título

CDD 333.95

ISABELLE CRISTINA SANTOS MAGALHÃES

POLINIZAÇÃO E SUA INFLUÊNCIA NA PRODUÇÃO DE CULTURAS DE CUCURBITACEAE

Tese defendida em: 28 / 05 / 2024

Presidente da Banca/Orientadora:

____

Profa. Dra. Cibele Cardoso de Castro

Universidade Federal do Agreste de Pernambuco – Orientadora

Banca Examinadora:

____

Profa. Dra. Kayna Agostini

Universidade Federal de São Carlos - Membro externo

____

Prof. Dr. Marcelo de Oliveira Milfont

Universidade Federal do Agreste de Pernambuco - Membro externo

____

Dr. Gudryan Jackson Barônio

Universidade de São Paulo- Membro externo

____

Prof. Dr. Natan Messias de Almeida

Universidade Federal Rural de Pernambuco - Membro interno

RECIFE – PE

2024

Dedico este trabalho a Deus e à Virgem Maria Santíssima, por toda força, coragem e proteção.

AGRADECIMENTOS

A Deus, agradeço eternamente por sua bondade e amor concedidos a cada amanhecer. E à Nossa Senhora, por ser o meu refúgio e fortaleza nessa jornada.

Aos meus pais, Manoel e Joseane, por todo amor, carinho, incentivo, ensinamentos, confiança, dedicação, compreensão e educação que me ofertaram. Agradeço, também, por sempre acreditarem nos meus sonhos e batalharem comigo para a sua concretização. E à minha irmã Emanuelle, por seu incentivo, carinho e apoio.

Ao meu esposo, Alison, pelo companheirismo, apoio, amor, dedicação, compreensão nos momentos de distância, e por ser o meu maior incentivador. Te amo!

À Universidade Federal Rural de Pernambuco pela oportunidade de realização do curso de Pós-Graduação em Biodiversidade.

À CAPES pela concessão da bolsa de estudos e apoio financeiro via PROAP.

À minha orientadora, Cibele Cardoso de Castro, por todo apoio, confiança, compreensão e ensinamentos compartilhados.

Aos produtores rurais agroecológicos, Erivane, Rosineide, Getúlio e Raminho, pela disponibilidade de suas propriedades para execução dos experimentos de campo.

À Universidade Federal do Agreste de Pernambuco pela disponibilização da área de estudo e da infraestrutura durante a coleta de dados de campo e laboratório.

Ao Núcleo de Estudos em Polinização Agrícola (NEPAG), especialmente Gerlayne e Marcelo, que contribuíram diretamente na realização dos experimentos de campo e laboratório.

À professora Daniele pela coorientação das análises de antioxidantes e à aluna Girlaine pelo auxílio durante a execução das análises em laboratório.

E a todos que torceram por essa conquista e de alguma forma contribuíram na realização desta tese.

LISTA DE FIGURAS

CAPÍTULO I. Similar flowers, different pollinators: worldwide pollination relations of Cucurbitaceae crops

Figure 1. Flowchart of the selection of studies on Cucurbitaceae crops pollination adapted from PRISMA 2020. 26

Figure 2. Global distribution of studies on Cucurbitaceae crops pollination 29

Figure 3. Number of studies that focused on floral visitation of cucurbits of economic importance along decades in the world. 59

Figure 4. Structure of the modular meta-network representing Cucurbitaceae crops and their pollinators global. Squares: plants; circles: pollinators; Clan: *Citrullus lanatus* (watermelon); Cmel: *Cucumis melo* (melon); Cpep: *Cucurbita pepo* (zucchini); Sedu: *Sechium edule* (chayote); Cmax: *Cucurbita maxima* (pumpkin); Laeg: *Luffa aegyptiaca* (sponge gourd); Carg: *Cucurbita argyrosperma* (gourd-neck); Cmos: *Cucurbita moschata* (squash); Lcyl: *Luffa cylindrica* (sponge gourd); Mcha: *Momordica charantia* (bitter gourd); Csat: *Cucumis sativus* (cucumber); Cang: *Cucumis anguria* (gherkins); Lsic: *Lagenaria siceraria* (bottle gourd); Lacu: *Luffa acutangula* (ridge gourd); MH: *Xenoglossa kansensis*; NH: *Apis florea* and *Apis mellifera*; C: *Apis cerana*, *Apis dorsata*, *Ceratina smaragdula*, *Peponapis limitis*, *Tetragonula iridipennis*, *Xylocopa aestuans*, *Xylocopa pubescens* and *Myrmica rubra*. And the floral visitor species (numbers) are defined in the supplementary material...34

Figure 5. Correlation plot showing the association and disassociation power of the pollinator groups and eight modules detected in the meta-net of interactions between Cucurbitaceae crops and pollinators around the world. Positive values in green indicate attraction/association, and negative values in pink indicate disassociation with the module 36

Figure 6. Roles of species in the global meta-network of interactions between Cucurbitaceae crops and pollinators according to their degree within the module (z) and connectivity between modules (c) of each species in each group. Following Dormann and Strauss (2014), we

calculated expected c and z values using null models based on the original networks, and used 95% quantiles as critical values. 37

CAPÍTULO II. Pollination of zucchini (*Cucurbita pepo* L., Cucurbitaceae): helping production improvement through a global review of research approaches, distribution of pollinators and identification of knowledge gaps

Figure 1. Flowchart of selection of pollination studies on zucchini (*Cucurbita pepo* L., Cucurbitaceae) adapted from PRISMA 2020. 88

Figure 2. Distribution of pollination studies in zucchini (*Cucurbita pepo* L., Cucurbitaceae) and its influence on production. (A) Study sites sampled worldwide. (B) Number of studies conducted over the decades. (C) Number of studies in tropical and temperate areas carried out in different environments. Open: plantations in the field; Closed: greenhouses; Both: both situations. 89

Figure 3. Distribution of the global literature on zucchinni (*Cucurbita pepo* L., Cucurbitaceae) floral visitors divided into taxonomic groups for the nine main genera and the four main orders. The number of studies performed with each genus is indicated by the fill color in the countries. The "other genera" consist of 28 genera, with the taxonomic orders indicated by fill color, consistent in the panel. The point size represents the frequency of mentioned studies 90

Figure 4. Interaction network between pollinators of zucchini (*Cucurbita pepo* L., Cucurbitaceae) and geographical distribution of studies. Circles represent pollinators and squares countries. The thickness of the line is proportional to the number of studies carried out with pollinators in each country. 91

CAPÍTULO III. Bee pollination increases fruit set and antioxidant activity in pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae)

Figure 1. Frequency of floral visits in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil. A, B, C: Areas I, II and II, respectively. 115

Figure 2. Effect of floral display on total frequency of floral visits (A), frequency of *Apis mellifera* (B) and *Trigona spinipes* (C) in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil. 116

Figure 3. Effect of the number of visits by all floral visitors (A), by *Trigona spinipes* (B) and by *Apis mellifera* (C) on production quality in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil. 117

Figure 4. Antioxidant activity of pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) determined by $ABTS^{+}$ assays in fruits resulted from natural (NP) and cross (CP) pollination experiments. 118

Figure 5. Relationship between number of seeds and fruit weight in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil. 118

LISTA DE TABELAS

CAPÍTULO I. Similar flowers, different pollinators: worldwide pollination relations of Cucurbitaceae crops

Table 1. Number (and percentage of the total number of pollinator species recorded) of pollinator species of different taxonomic groups recorded in Cucurbitaceae crops worldwide. Blatto: Blattodea; Coleop: Coleoptera; Lepidop: Lepidoptera; Mantod: Mantodea; Odonat: Odonata; Orthop: Orthoptera; Collet: Colletidae; Eumen: Eumenidae; Formic: Formicidae; Halict: Halictidae; Megach: Megachilidae; Vesp: Vespidae. 30

CAPÍTULO III. Bee pollination increases fruit set and antioxidant activity in pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae)

Table 1. Floral visitors observed in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations of a semiarid region of NE Brazil. 112

Table 2. Influence of the total number of visits, visits by *A. mellifera*, visits by *T. spinipes* and the joint effect of visitation by both species on pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) production parameters in three plantations of a semiarid region of NE Brazil. $\chi 2$, chi-square values results from analysis of variance. *Significant effect on a given response variable ($p < 0.05$). 113

Table 3. Effect of pollination treatments (natural and cross pollinations) and seed number on yield quantity and quality parameters in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations of a semiarid region of NE Brazil. Fruit set per treatment is given as a percentage. Mean values (± SD) per treatment are presented for the other response variables. $\chi 2$, chi-square values, analysis of variance results. *Significant effect on a given response variable ($p < 0.05$). 113

SUMÁRIO

INTRODUÇÃO ... **14**
REFERÊNCIAS ... **16**
CAPÍTULO I. Similar flowers, different pollinators: worldwide pollination relations of Cucurbitaceae crops ... **20**
Abstract ... 21
Introduction ... 23
Materials and methods ... 25
Results ... 27
Discussion ... 38
Conclusions ... 44
References ... 45
CAPÍTULO II. Pollination of zucchini (*Cucurbita pepo*, Cucurbitaceae): helping production improvement through a global review of research approaches, distribution of pollinators and identification of knowledge gaps ... **66**
Abstract ... 67
Introduction ... 69
Materials and methods ... 70
Results ... 71
Discussion ... 73
Conclusions ... 77
References ... 79
CAPÍTULO III. Bee pollination increases fruit set and antioxidant activity in pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) ... **92**
Abstract ... 93
Introduction ... 94
Materials and methods ... 95
Results ... 99
Discussion ... 100
Conclusions ... 103
References ... 104

RESUMO

A produção de muitas culturas agrícolas depende de polinizadores, especialmente de abelhas, consideradas as principais responsáveis pelo rendimento das culturas, influenciando o tamanho, peso, e a composição química dos frutos e sementes. No entanto, embora se saiba de sua importância, a presença de insetos polinizadores nas culturas vem reduzindo, ameaçando a segurança nutricional global. Com isso, as culturas agrícolas que são mais dependentes de polinizadores são consequentemente as mais afetadas, dentre as da família Cucurbitaceae, como a abóbora (*Cucurbita moschata*), por causa da monoicia que apresentam. Apesar da importância da família na produção agrícola mundial, não há trabalhos que integrem estatisticamente dados de interação das culturas de Cucurbitaceae e seus polinizadores, nem indiquem as lacunas de conhecimento. Além disto, tendo em vista a importância dos polinizadores na produção de abóbora, investigações sobre a influência da polinização na produção são essenciais para auxiliar ações de manejo e conservação de polinizadores. O objetivo deste trabalho foi conhecer a tendência dos estudos e das interações planta-polinizador de cucurbitáceas de importância econômica em escala global, integrar dados globais sobre a polinização da abobrinha (*Cucurbita pepo*) e avaliar a relação entre exposição floral, frequência de visitas, quantidade e qualidade da produção agrícola, incluindo análises de antioxidantes de frutos, usando como modelo o cultivo de abóbora (*Cucurbita moschata*). A tese de doutorado está estruturada em três capítulos. No primeiro capítulo, foi realizado uma revisão sistemática da literatura e a partir dos dados de visitantes florais, construída uma meta-rede. A meta-rede foi altamente modular, com a maioria das espécies sendo periféricas, o hub de módulo foi *Xenoglossa kansensis* e os hubs de rede foram *Apis florea* e *A. mellifera.* A formação de módulos não pode ser explicada exclusivamente por características funcionais das espécies, pois embora as flores sejam semelhantes na cor, elas diferem em outros atributos, explicando a grande diversidade de insetos observada. Nossos dados reforçam a necessidade de apoiar os polinizadores nativos, contribuindo para a mitigação da crise global dos polinizadores. No segundo capítulo, através de uma revisão sistemática, analisamos a distribuição dos estudos, construímos uma rede de países e polinizadores e comparamos dados sobre a eficiência de polinizadores específicos. Os estudos foram realizados em quase todos os continentes, onde a maioria dos estudos investigou a frequência e diversidade de visitantes florais. As flores de abobrinha alimentaram 116 espécies de polinizadores, principalmente abelhas. Muitos países possuíam grupos quase exclusivos de polinizadores nativos, entre os quais é possível encontrar espécies eficientes e manejáveis, capazes de substituir polinizadores exóticos. Os dados aqui compilados ajudarão no desenvolvimento e aprimoramento de estratégias para o manejo e conservação dos polinizadores. Além disso, estudos futuros sobre a influência dos polinizadores nos aspectos químicos dos frutos e na germinação das sementes são necessários. No terceiro capítulo, verificamos a biologia floral, realizamos observações focais e conduzimos tratamentos de polinização (natural e cruzada). Foram registradas sete espécies de visitantes, sendo *A. mellifera* e *T. spinipes* as mais frequentes. A frutificação resultante da polinização natural foi maior que a da polinização cruzada e apresentaram maior atividade antioxidante dos frutos. Além disso, o número de sementes esteve positivamente relacionado com o peso dos frutos. Assim, as abelhas foram os principais polinizadores da abóbora produzida na região semiárida do Nordeste do Brasil, com destaque para *T. spinipes* e *A. mellifera*, o que influenciou positivamente no peso dos frutos. Além disso, os polinizadores favoreceram o potencial antioxidante dos frutos.

Palavras-chave: Hortaliça; Polinizadores; Abelhas; Visitantes florais; *Cucurbita*.

ABSTRACT

The production of many agricultural crops depends on pollinators, especially bees, considered primarily responsible for crop yields, influencing the size, weight, and chemical composition of fruits and seeds. However, although its importance is known, the presence of pollinating insects in crops has been decreasing, threatening global nutritional security. As a result, agricultural crops that are more dependent on pollinators are consequently the most affected, among those in the Cucurbitaceae family, such as pumpkin (*Cucurbita moschata*), due to their monoecy. Despite the importance of the family in global agricultural production, there are no studies that statistically integrate data on the interaction of Cucurbitaceae crops and their pollinators, nor indicate gaps in knowledge. Furthermore, given the importance of pollinators in pumpkin production, investigations into the influence of pollination on production are essential to assist with pollinator management and conservation actions. The objective of this work was to understand the trend of studies and plant-pollinator interactions of cucurbits of economic importance on a global scale, integrate global data on the pollination of zucchini (*Cucurbita pepo*) and evaluate the relationship between floral display, frequency of visits, quantity and quality of agricultural production, including analysis of fruit antioxidants, using pumpkin cultivation (*Cucurbita moschata*) as a model. The doctoral thesis is structured into three chapters. In the first chapter, a systematic review of the literature was carried out and, based on data from floral visitors, a meta-network was constructed. The meta-network was highly modular, with most species being peripheral, the module hub was *Xenoglossa kansensis* and the network hubs were *Apis florea* and *A. mellifera*. The formation of modules cannot be explained exclusively by functional characteristics of the species, because although the flowers are similar in color, they differ in other attributes, explaining the great diversity of insects observed. Our data reinforces the need to support native pollinators, contributing to mitigating the global pollinator crisis. In the second chapter, through a systematic review, we analyze the distribution of studies, build a network of countries and pollinators and compare data on the efficiency of specific pollinators. Studies have been carried out on almost every continent, where most studies have investigated the frequency and diversity of floral visitors. Zucchini flowers fed 116 species of pollinators, mainly bees. Many countries had almost exclusive groups of native pollinators, among which it is possible to find efficient and manageable species, capable of replacing exotic pollinators. The data compiled here will help in the development and improvement of strategies for the management and conservation of pollinators. Furthermore, future studies on the influence of pollinators on the chemical aspects of fruits and seed germination are necessary. In the third chapter, we verify floral biology, carry out focal observations and conduct pollination treatments (natural and cross). Seven species of visitors were recorded, with *A. mellifera* and *T. spinipes* being the most frequent. The fruit set resulting from natural pollination was greater than that from cross pollination and presented greater antioxidant activity of the fruits. Furthermore, the number of seeds was positively related to fruit weight. Thus, bees were the main pollinators of pumpkin produced in the semi-arid region of Northeast Brazil, with emphasis on *T. spinipes* and *A. mellifera*, which positively influenced the weight of the fruits. Furthermore, pollinators favored the antioxidant potential of the fruits.

Keywords: Crops; Pollinators; Bees; Floral visitors; *Cucurbita*

INTRODUÇÃO

Mais de 75% das espécies cultivadas para consumo humano necessitam da presença dos agentes polinizadores para a formação de seus frutos e sementes, pois influenciam positivamente em diversas características comerciais, garantindo maior valor econômico no mercado (Klein et al., 2007; Gemmill-Herren, 2016). As abelhas são as principais responsáveis pelo rendimento das culturas, influenciando o conjunto e a qualidade dos frutos e sementes (tamanho, peso e composição química; Garibaldi et al., 2013; Klatt et al., 2014; Giannini et al., 2015). Apesar de tal importância, é globalmente aceito que a presença de insetos polinizadores nas culturas vem reduzindo em decorrência de múltiplos fatores antropogênicos, como uso excessivo de agrotóxicos, introdução de patógenos, mudanças climáticas e principalmente ameaças causadas pelas mudanças de habitat, como o desmatamento da vegetação nativa (Potts et al., 2010), colocando em risco a segurança nutricional global (Eilers et al., 2011; Smith et al., 2015; IBPES, 2016). Culturas agrícolas que são mais dependentes de polinizadores, são consequentemente as mais afetadas (Klein et al., 2018), dentre elas destacam-se aquelas da família Cucurbitaceae.

A família Cucurbitaceae compreende 97 gêneros e, aproximadamente, 980 espécies que são distribuídas nas regiões tropicais e subtropicais do mundo, no Brasil existem 30 gêneros e 157 espécies distribuídas em todas as regiões (Agbagwa et al., 2007; Schaefer & Renner, 2011). A domesticação de várias espécies de importância agrícola começou a 11.000 anos a.C. no Novo Mundo e na Ásia em tempos pré-colombianos, e mais recente na África (Larson et al., 2014; Chomicki et al., 2019), sendo o gênero *Cucurbita* com a maior distribuição e adaptação a ambientes perturbados (Kistler el al., 2015), e consequentemente consideradas as primeiras espécies domesticadas (Cutler & Whitaker, 1961). A maioria das espécies possuem grande importância econômica em virtude dos seus frutos e sementes apresentarem alto valor nutricional, sendo utilizados como fonte alimentar. Além disso, alguns frutos secos podem ser empregados na confecção de utensílios, e outras espécies na fabricação de fármacos (Amadou & Bako, 2018; Maja et al., 2022). Dentre as espécies mais cultivadas mundialmente destacam-se *Cucurbita moschata* Duchesne (abóbora), *Cucurbita maxima* L. (jerimum), *Cucurbita pepo* L. (abobrinha), *Cucumis melo* L. (melão), *Cucumis sativus* L. (pepino), *Citrullus lanatus* (Thunb.) Matsum. & Nakai (melancia), *Sechium edule* (Jacq.) Sw. (chuchu) e *Cucumis anguria* L. (maxixe).

A produção mundial de *Cucurbita* foi de cerca de 35 milhões de toneladas, cultivadas aproximadamente em dois milhões de hectares, com a maior parte da produção concentrada na China e na Índia (FAO, 2024). Estima-se que o valor econômico dos serviços de polinização em todas as cucurbitáceas seja em torno de dois bilhões de reais (Wolowski et al., 2019). A abóbora (*C. moschata*) é uma das hortaliças de maior importância socioeconômica do gênero *Cucurbita*, em grande parte devido aos seus frutos e sementes apresentarem alto valor nutritivo, sendo a polpa dos frutos rica em alto teor de carotenoides, compostos polifenólicos, componentes minerais e vitamina C, assim possuindo alto potencial de antioxidante (Kulczyński et al., 2020). Estudos recentes indicam que antioxidantes oriundos de vegetais e frutas contribuem para reduzir o risco de desenvolvimento de diversas doenças cardiovasculares (Aune et al., 2018), neurodegenerativas (Li et al., 2012), entre outras.

As plantas de cucurbitáceas possuem diferentes tipos de sistema reprodutivo, podendo ser em sua grande maioria monoica e outras andromonoicas (Free, 1993; Delaplane and Mayer, 2000). As plantas da abóbora são monoicas e protândricas dentro da planta, ou seja, a abertura das flores masculinas iniciando primeiro do que as femininas, o que ocorre duas a três semanas depois. Apresentam tamanho relativamente grande, com corola campanulada amarelo brilhante (Agbagwa et al., 2007). As flores masculinas produzem grande quantidade de grãos de pólen, com textura pegajosa e sendo facilmente transportado por insetos polinizadores até a flor feminina (Rech et al., 2014). Devido a esses aspectos, as flores necessitam obrigatoriamente de visitantes florais para obter polinização e produção de frutos bem-sucedidos (Delaplane, 2000). As flores de *Cucurbita*, além de serem grandes e vistosas, oferecem recompensas relativamente ricas em pólen e néctar aos polinizadores que as visitam (Nicodemo et al., 2009). As plantas de melão podem ser andromonoicas, ou seja, têm flores estaminadas e hermafroditas na mesma planta, com disponibilidade de pólen e néctar (McGregor, 1976; Delaplane and Mayer, 2000). Mesmo as flores sendo hermafroditas, a presença dos polinizadores é fundamental, visto que as anteras se posicionam para fora, e os grãos de pólen tendem a cair nas pétalas (Free, 1993). Os principais polinizadores são abelhas das famílias Apidae (*Apis mellifera*, *Melipona* spp. *Trigona spinipes*), Adrenidae (*Oxaea flavescens*, *Bombus morio*), e besouros da família Chrysomelidae (*Diabrotica* spp.; Giannini et al., 2015).

A produção agrícola de cucurbitáceas depende em grande parte das interações com os polinizadores, estudos com abordagem de meta-rede de polinização permitem a identificação de polinizadores importantes na coesão e estabilidade da rede, evitando a formação de comunidades isoladas (González et al., 2010; Tilianakis & Morris, 2017). Portanto, tais estudos também permitem identificar grupos-alvo de polinizadores para conservação e manejo (Emer et al., 2018; Librán-Embid et al., 2021), culturas agrícolas vulneráveis, bem como lançar luz sobre culturas cuja polinização é pouco conhecida (Paulino et al., 2021). Considerando que culturas de Cucurbitaceae constituem importantes componentes da produção agrícola mundial e que são muito dependentes de polinizadores para a produção, trabalhos que integrem estatisticamente dados de polinização são importantes para a identificação das relações ecológicas entre as culturas mais importantes de Cucurbitaceae e seus polinizadores, porém escassos. Além disto, tendo em vista a importância dos polinizadores na produção de abóbora, investigações sobre a influência da polinização na produção da abóbora são essenciais para auxiliar ações de manejo. O objetivo geral desse trabalho foi conhecer as interações registradas entre cucurbitáceas de importância econômica e visitantes florais em escala global, integrar dados globais sobre a polinização da abobrinha (*Cucurbita pepo*) e avaliar a influência da polinização sobre a polinização e a produção de abóbora (*Cucurbita moschata*) no semiárido do NE do Brasil.

REFERÊNCIAS

Agbagwa, I. O., Ndukwu, B. C., Mensah, S. I. (2007). Floral Biology, Breeding System, and Pollination Ecology of *Cucurbita moschata* (Duch. ex Lam) Duch. ex Poir. Varieties (Cucurbitaceae) from Parts of the Niger Delta, Nigeria. *Turkish Journal of Botany*, 31, 451–458.

Amadou, I., Bako, A. T. (2018). Perception of *Lagenaria siceraria* Fruits on Their Nutritive, Therapeutic and Organoleptic Values. *American Journal of Food, Nutrition and Health*, 3(3), 26–30.

Aune, D., Keum, N., Giovannucci, E., Fadnes, L. T., Boffetta, P., Greenwood, D. C., Tonstad, S., Vatten, L. J., Riboli, E., Norat, T. (2018). Dietary intake and blood concentrations of antioxidants and the risk of cardiovascular disease, total cancer, and all-cause mortality: A systematic review and dose-response meta-analysis of prospective studies. *American Journal of Clinical Nutrition*, 108(5), 1069–1091. https://doi.org/10.1093/ajcn/nqy097

Chomicki, G., Schaefer, H., Renner, S. S. (2020). Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. *New Phytologist*, 226, 1240-1255. https://doi.org/10.1111/nph.16015

Cutler, H. C., Whitaker, T. W. (1961). History and Distribution of the Cultivated Cucurbits in the Americas. *Source: American Antiquity*, 26(4), 469-485.

Delaplane, K. S., Mayer, D. F. (2000). Crop pollination by bees. Nova York: CABI Publishing. 344 p.

Eilers, E. J., Kremen, C., Smith Greenleaf, S., Garber, A. K., Klein, A. M. (2011). Contribution of Pollinator-Mediated Crops to Nutrients in the Human Food Supply. *PLoS One*, 6, e21363. https://doi.org/10.1371/journal.pone.0021363

Emer, C., Galetti, M., Pizo, M. A., Guimarães Jr., P. R., Moraes, S., Piratelli, A. & Jordano, P. (2018). Seed-dispersal interactions in fragmented landscapes – a metanetwork approach. *Ecology Letters, 21*, 484-493. https://doi.org/10.1111/ele.12909

Food and Agriculture Organization of The United Nations- FAO STATE. 2021. In: FAO STATE [Internet]. Rome: FAO STATE 2024.

Free, J.B. (1993). Insect Pollination of Crops. London, U.K.: Academic Press, 684pp.

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A. et al. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, 339(6127), 1608–1611. https://doi.org/10.1126/science.123020

Gemmill-Herren, B. (2016). Pollination services to agriculture - Sustaining and enhancing a key ecosystem service (Food and Agriculture Organization of the United Nations). Routeledge, New York.

Giannini, T. C., Boff, S., Cordeiro, G. D., Cartolano Jr, E. A. Veiga, A. K., Imperatriz-Fonseca, V. L., Saraiva, A. M. (2015). Crop pollinators in Brazil: a review of reported interactions. *Apidologie*, 46, 209–223. https://doi.org/10.1007/s13592-014-0316-zv

González, A. M. M., Dalsgaard, B. & Olesen, J. M. (2010). Centrality measures and the importance of generalist species in pollination networks. *Ecological Complexity*, *7*, 36-43. https://doi.org/10.1016/j.ecocom.2009.03.008

IPBES. (2016). The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination, and food production. Potts SG, Imperatriz-Fonseca VL, Ngo HT (eds). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn. Germany. 552 pages.

Kistler, L., Newsom, L. A., Ryan, T. M., Clarke, A. C., Smith, B. D., Perry, G. H. (2015). Gourds and squashes (*Cucurbita* spp.) adapted to megafaunal extinction and ecological anachronism through domestication. *Proceedings of the National Academy of Sciences, USA*, 112, 15107–15112. https://doi.org/10.1073/pnas.1516109112

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E. et al. (2014) Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences*, 281, 24–40. https://doi.org/10.1098/rspb.2013.2440

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Scharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, 274, 303–313. https://doi.org/10.1098/rspb.2006.3721

Kulczyński, B., Sidor, A., Gramza-Michałowska, A. (2020) Antioxidant potential of phytochemicals in pumpkin varieties belonging to *Cucurbita moschata* and *Cucurbita pepo* species. *CyTA - Journal of Food*, 18(1), 472–484, https://doi.org/10.1080/19476337.2020.1778092

Larson, G., Piperno, D. R., Allaby, R. G., Purugganan, M. D., Andersson, L., Arroyo-Kalin, M., Barton, L., Vigueira, C. C., Denham, T., Dobney, K. et al. (2014). Current perspectives and the future of domestication studies. *Proceedings of the National Academy of Sciences, USA*, 11, 6139–6146. https://doi.org/10.1073/pnas.1323964111

Li, F. J., Shen, L., Ji, H. F. (2012). Dietary intakes of vitamin E, vitamin C, and β-carotene and risk of Alzheimer's disease: A meta-analysis. *Journal of Alzheimers Disease*, 31(2), 253–258. https://doi.org/10.3233/JAD-2012-120349

Librán-Embid, F., Grass, I., Emer, C., Ganuza, C. & Tscharntke, T. (2021). A plant–pollinator metanetwork along a habitat fragmentation gradient. *Ecology Letters*, *24*, 2700-2712. https://doi.org/10.1111/ele.13892

Maja, D., Mavengahama, S., Mashilo, J. (2022). Cucurbitacin biosynthesis in cucurbit crops, their pharmaceutical value and agricultural application for management of biotic and abiotic stress: A review. *South African Journal of Botany*, 145, 3–12. https://doi.org/10.1016/j.sajb.2021.08.044

Mcgregor, S. E. (1976). Insect Pollination of Cultivated Crop Plants. Washington, DC: United States Department of Agricultural Research Service, 496pp.

Nicodemo, D., Couto, R. H. N., Malheiros, E B., Jong, D. (2009). Honey bee as an effective pollinating agent of pumpkin. *Scientia Agricola*, 66(4), 476–480. https://doi.org/10.1590/S0103-90162009000400007

Paulino, C. S., Souza, C. S., Santa-Martinez, E., Almeida, N. M. & Castro, C. C. (2021). Global meta-network of legume crops and floral visitors reveals abundance of exotic bees. *Journal of Applied Entomology*, *146*, 252-261. https://doi.org/10.1111/jen.12956

Potts, S. G., Biesmeijer, J. C, Kremen, C., Neumann, P. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution*, 25(6), 345–353. https://doi.org/10.1016/j.tree.2010.01.007

Rech, A. R., Agostini, K., Oliveira, P. E., Machado, I. C. (2014). Biologia da polinização. Rio de Janeiro: Projeto cultural, p. 527.

Smith, M., Singh, G., Mozaffarian, D., Myers, S. (2015). Effects of decreases of animal pollinators on human nutrition and global health: a modelling analysis. *The Lancet*, 386, 1964–1972. https://doi.org/10.1016/S0140-6736(15)61085-6

Schaefer, H., Renner, S. S. (2011). Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). *Taxonomy*, 60(1), 122–138. https://doi.org/10.1002/tax.601011

Wolowski, M. et al. (2019). Relatório temático sobre polinização, polinizadores e produção de alimentos no Brasil. 1 ed. São Carlos, SP: Editora Cubo.

CAPÍTULO I

Similar flowers, different pollinators: worldwide pollination relations of Cucurbitaceae crops

Trabalho submetido à revista Plant Biology em dezembro de 2023

Similar flowers, different pollinators: worldwide pollination relations of Cucurbitaceae crops

Isabelle Cristina Santos Magalhães[1]; Kleber Régis Santoro[2]; Cibele Cardoso Castro[1,2*]

[1] Programa de Pós-Graduação em Biodiversidade, Universidade Federal Rural de Pernambuco, Recife, Pernambuco, Brazil; smagalhaes.isabelle@gmail.com

[2] Universidade Federal do Agreste de Pernambuco, Garanhuns, Pernambuco, Brazil; krsantoro01@gmail.com, cibele.castro@ufape.edu.br

*Corresponding author

Abstract. Cucurbitaceae is an economically important plant family whose crop production is essentially dependent on pollinators. Large-scale relations between plants and pollinators may be studied with a meta-network approach. This study aims to understand how the ecological relationships among the most important Cucurbitaceae crops and their pollinators are organized globally and indicate gaps in knowledge by using a meta-network approach. We aim to answer the following questions: (1) How are interactions between plants and pollinators structured? (2) What are the roles of the crops and pollinator species in structuring the network? (3) Which functional traits are responsible for the formation of the modules? (4) Which pollinators are particularly relevant for which plants? (5) What traits do hub pollinators species have? Plants and pollinators were obtained through a systematic literature review. The modularity and the role played by crops and pollinators species were calculated, and a comparison of the pollinator composition among modules was made. In the highly modular meta-network, most pollinator species were peripheral (few links in their own module). Most connector species (link several modules) belong to bee genera *Apis, Ceratina*, *Peponapis*, *Tetragonula* and *Xylocopa*. The only module hub (generalist) was the bee *Xenoglossa kansensis*, which is highly specialized in collecting *Cucurbita* pollen. The generalist bees *Apis florea* and *A. mellifera* were the network hubs (supergeneralists). Apidae, Halictidae and Megachilidae bees were the most structurally important pollinators for all plants, especially for chayote, gherkin, melon, sponge gourds and squash-neck. Modules formation cannot be explained exclusively by functional traits of plants species. Although flowers are similar in color, they differ in other attributes, explaining the high diversity of insects observed. Gherkin (*Cucumis anguria*), squash (*Cucurbita moschata*), chayote (*Sechium edule*), and sponge gourd (*Luffa aegyptiaca* and *Luffa cylindrica*) need further pollination studies. Our data reinforce the necessity of supporting native pollinators, contributing to mitigation of the global pollinator crisis. Investigations on the differential efficiency of native, manageable pollinators are necessary.

Keywords: crop pollination, ecosystem services, network, modularity.

Introduction

Cucurbitaceae is the fourth most economically important plant family, with an annual world production estimated at more than 340 billion USD (FAOSTAT, 2022). Plantations occupy about 12 million hectares distributed in tropical and temperate regions, which produce more than 390 million tons of fruits annually (FAOSTAT, 2022). Such economic importance is due to its fruits and seeds which provide a rich source of nutrients for human and animal food (Patel & Rauf, 2017), and have important secondary metabolites (triterpenoids) of medicinal use (Shah et al., 2014; Barghamdi et al., 2016; Amadou & Bako, 2018; Chomicki et al., 2020). Among the most cultivated species are the pumpkins and squash (*Cucurbita moschata* Duchesne, *Cucurbita maxima* L., *Cucurbita ficifolia* Bouché, *Cucurbita argyrosperma* Huber, and *Cucurbita pepo* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], melon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.). The production of Cucurbitaceae crops is essentially pollinator dependent (Klein et al., 2018), as most species are monoecious producing imperfect male and female flowers on the same individual (Chomicki et al., 2020).

Insect pollination is necessary for Cucurbitaceae crops' production because their large (100-200 µm) and sticky pollen grains covered by a thick layer of oily pollenkit cannot be removed by the wind (Rech et al., 2014). The flowers provide large amounts of pollen and nectar with high sugar concentration throughout the flowering period (Vidal et al., 2010), supporting a wide diversity of pollinators such as wasps, ants, flies, beetles, bees, and butterflies (Ekeke et al., 2018; Wolowski et al., 2019). The most recorded pollinators are bees especially from the families Apidae and Halictidae (Enríquez et al., 2015; Gómez et al., 2016) and butterflies (Balachandran et al., 2017; Patil & Jagdale, 2021). Pollinators are attracted to cucurbits by their large and showy flowers, with mainly bright yellow petals (Nepi & Pacini, 1993) excep chayote and bottle gourd, which are white (Saade, 1996; Morimoto et al., 2005), and their mild odor dispersed over long distances (Agbagwa et al., 2007).

Although most Cucurbitaceae crops have similar yellow flowers, they differ in several floral attributes. Corolla diameter varies from 2.5-3.5 cm in cucumber (Malepszy & Niemirowicz-Szczytt, 1991), to 14-20 cm in pumpkins (Sinu et al., 2017). Anthesis duration varies, from a few hours during the day e.g., six hours in pumpkins (Pinkus-Rendon et al., 2005), a few hours during the night e.g., 8-10 hours in botther gourd (Okunlola et al., 2022), 18 hours in gherkin (Carneiro Neto et al., 2018), or two days in chayote (Malerbo-Souza et al., 2023). The number of stamens may also vary from three for cucumber and watermelon (Bomfim et al., 2013) to five for pumpkins (Free, 1993). The distribution of floral resources may also differ among crops, as female flowers of the bitter gourd and bottle gourd do not secrete nectar (Teppner, 2004; Lenzi et al., 2005) whereas other crops produce copious amounts of nectar (Nepi et al., 2001). This diversity of floral attributes explains the high diversity of floral visitors observed in the family.

As observed in more than 70% of crops, insect pollination not only improves the quantity and quality of Cucurbitaceae production (Klatt et al., 2014; Potts et al., 2016; Fijen et al., 2018), but makes it actually possible, because of the monoecy (Donoso & Murúa, 2021; Khalifa et al., 2021), However, world food production is potentially threatened by the pollinators' decline and consequent pollination performance (Potts et al., 2010; IBPES, 2016), threatening global nutritional security and the stability of ecosystems (Eilers et al., 2011; Smith et al., 2015; IBPES, 2016).

Strategies for the promotion and conservation of pollinators can be better targeted with the aid of large-scale pollination studies that include the meta-network approach, such as in grass communities (Danieli-Silva et al., 2012), of tropical species from the Peruvian Andes (Watts et al., 2016), urban environments (Nascimento et al., 2020), legume crops around the world (Paulino et al., 2021) and major crops in North America (Rondeau et al., 2022). Those studies show that pollination meta-networks may be modular (Olesen et al., 2007; Bascompte & Jordano, 2007). Modularity is a structural property of ecological networks; in modular networks, species from one module are linked together more strongly than with species from other modules (Olesen et al., 2007). Species of a network can be classified into different functional roles according to centrality metrics, which are related to their position within and between modules (Olesen et al., 2007; Martín González et al., 2012). Module hubs or generalists are species linked to many species within their own modules, connectors link several modules, peripherals or specialists have only a few links generally within their own module, and network hubs or super-generalists act as both connectors and module hubs (Olesen et al., 2007). Network hubs can play a key role in the pollination efficiency of various agricultural crops (Cagua et al., 2019). Despite the relevance of meta-network approach in pollination studies, it has some limitations related to bias regarding the allocation of studies, lack of identification of pollinator species, and the exclusion of studies in which interactions are mentioned rather casually.

Considering that cucurbits agricultural production largely depends on interactions with pollinators, studies with a pollination meta-network approach allow the identification of pollinators that are important in the cohesion and stability of the network, avoiding the formation of isolated communities (González et al., 2010; Tylianakis & Morris, 2017). Therefore, such studies also allow the identification of target groups of pollinators for conservation and management (Emer et al., 2018; Librán-Embid et al., 2021), vulnerable agricultural crops, as well as shedding light on crops whose pollination is little known (Paulino et al., 2021).

This study aims to understand how the ecological relationships among the most important Cucurbitaceae crops (pumpkins and squash, *Cucurbita moschata*, *Cucurbita maxima*, *Cucurbita argyrosperma* and *Cucurbita pepo*; watermelon, *Citrullus lanatus*; melon, *Cucumis melo*; cucumber, *Cucumis sativus*; bitter gourd, *Momordica charantia*; bottle gourd, *Lagenaria siceraria*; chayote, *Sechium edule*; gherkins, *Cucumis anguria*; ridge gourd, *Luffa acutangula*; sponge gourds, *Luffa aegyptiaca* and *Luffa cylindrica*) and their pollinators are organized globally and indicate gaps in knowledge by using a meta-network approach. We aim to answer the following questions: (1) How are interactions between plants and pollinators structured? (2) What are the roles of the crop and pollinator species in structuring the network? (3) Which functional traits are responsible for the formation of the modules? (4) Which pollinators are particularly relevant for which plants? (5) What traits do hub species have?

Materials and methods

Literature review

We conducted a systematic literature review on recorded interactions between Cucurbitaceae crops and pollinators using the databases Google Scholar (www.scholar.google.com), Scielo (www.scielo.org), Scopus (www.scopus.com) and Web of Science (www.webofknowledge.com), with year restriction (1960 to 2021). We used terms related to plant reproduction (i.e., pollination, pollinator, floral visitor, floral biology, breeding system) crossed with the scientific and the most used popular names of all cultivated species of Cucurbitaceae extracted from FAO (www.fao.org), i.e., bitter gourd (*Momordica charantia* L.), bottle gourd [*Lagenaria siceraria* (Molina) Standl.], chayote [*Sechium edule* (Jacq.) Sw.], cucumber (*Cucumis sativus* L.), gherkins (*Cucumis anguria* L.), gourd/squash/mogango-neck (*Cucurbita argyrosperma* Huber), melon (*Cucumis melo* L.), pumpkin (*Cucurbita maxima* Duchesne), ridge gourd [*Luffa acutangula* (L.) Roxb.], sponge gourds [*Luffa aegyptiaca* L. and *Luffa cylindrica* (L.) Durand & Durand], squash/pumpkin (*Cucurbita moschata* Duchesne ex Poir.), watermelon [*Citrullus lanatus* (Thunb.) Mansf.], zucchini/squash/pumpkin, (*Cucurbita pepo* L.). To obtain a greater probability of retrieving relevant studies, the following search string was used: ((“popular name” OR “scientific name”) AND (“pollination” OR “pollinator” OR “floral visitor” OR “floral biology” OR “breeding system”)).

Our inclusion criterion was that the study clearly stated that the floral visitors were pollinators or potential pollinators. We excluded papers whose title and abstract clearly indicated that the study did not contain pollination data (Fig. 1). From such studies, the year of publication, country of data collection, and identity of plants and pollinators were extracted. The scientific names of the pollinators were verified and updated using the Moure's Bee Catalog (moure.cria.org.br/) and the Global Names Resolver (resolver.globalnames.org/). Plants' scientific names were checked at SpeciesLink (http://inct.splink.org.br/) and Missouri Botanical Garden's Tropicos (http://www.tropicos.org). Only species level studies were used.

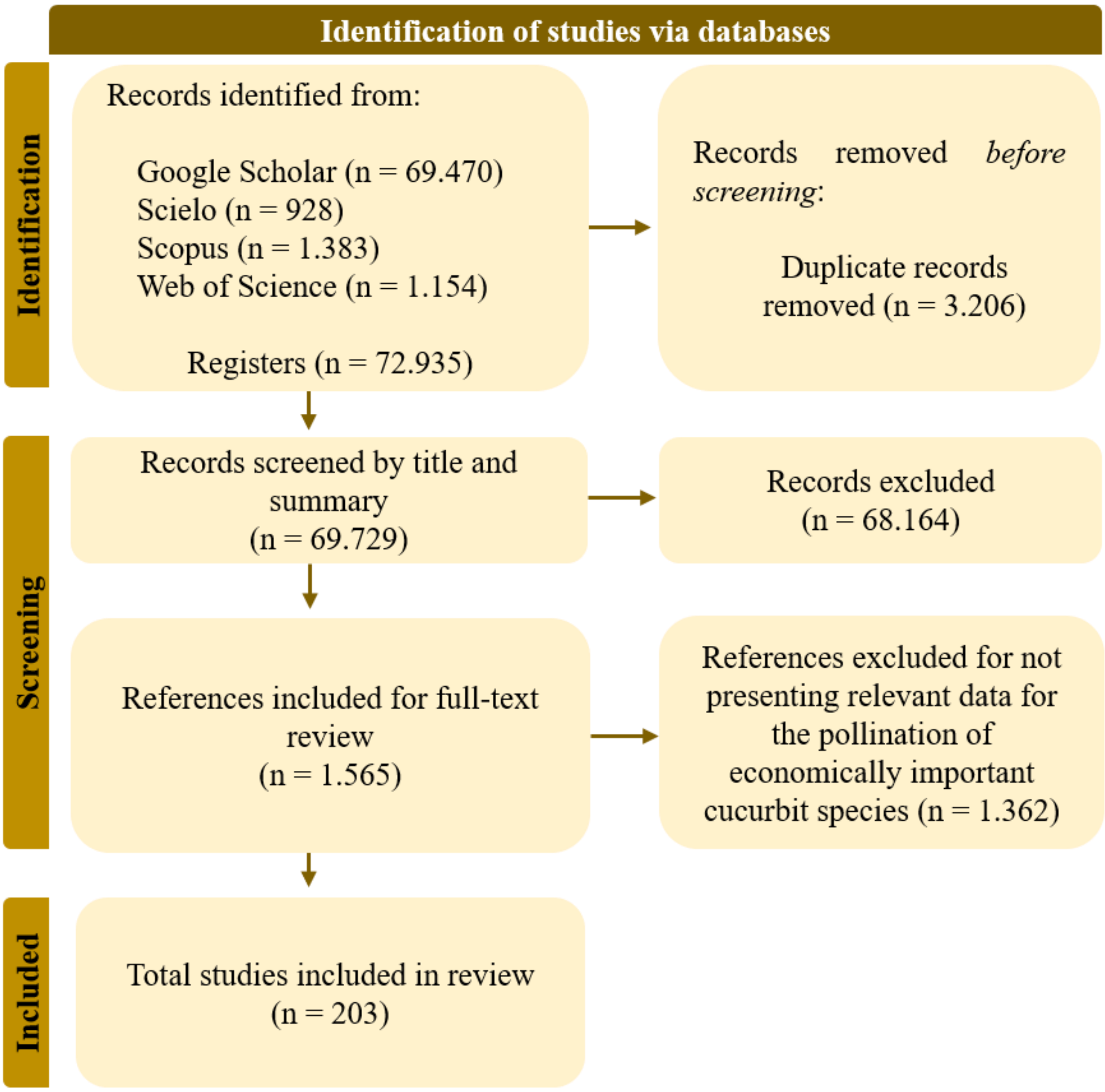

Figure 1. Flowchart of the selection of studies on Cucurbitaceae crops pollination adapted from PRISMA 2020.

Analyses

Through a binary matrix, the plant-pollinator interaction meta-network was constructed using the numbers 0 and 1 to represent, respectively, the absence or presence of interaction between pollinator species allocated in columns and plant species in rows (Araujo et al., 2018; Nascimento et al., 2020). To characterize the association between plants and pollinators, we conducted a modularity analysis of the meta-network. We observed the preferential interaction between partners within the modules, as well as identifying the species that are fundamental in structuring the entire network (Olesen et al., 2007; Araujo et al., 2018). Modularity was calculated with the LPAb+ algorithm (Beckett, 2016; Liu & Murata, 2010), in addition to the computeModules() function estimated in the bipartite package (Dormann et al., 2008), setting the number of steps to 10^9 and using default options. To verify stabilization, we performed modularity 1000 times. All analyzes performed in R Software (R Core Team, 2016).

As network metrics can be affected by the number of interacting species (Blüthgen et al., 2006; Fründ et al., 2016; Vizentin-Bugoni et al., 2016), the significance of modularity was evaluated by comparing it with a null template. In this meta-network, the swap.web binary null model was used similarly to that suggested by Vázquez et al. (2005), restricting connectance and marginal totals. From the 10,000 simulated values, the 95% confidence interval for the modularity metric (LPAb +) was estimated, and the metric value was considered significant when the confidence interval did not overlap. Finally, two indices related to species level were calculated: c (connectivity between modules) and z (degree of connectivity within the module). The index c quantifies the importance of a species as a connector of different modules, and z is the importance of the species in its own module (Olesen et al., 2007). According to c and z values, species were classified as module hub (or generalists, i.e., highly connected species linked to many species within their own module), connector (connected to several modules), peripheral (or specialists, i.e., have only a few links and mostly to species within their module) and network hub (or super generalists, i.e., act as connectors and module hubs), following the boundaries established by Olesen et al. (2007) and Dormann & Strauss (2014).

The composition of pollinators between modules was differentiated from a correlation matrix consisting of the orders of pollinators organized into functional groups (Olesen et al., 2007), using the corrplot package for module identity (Wei & Simko, 2017) and tested by a chi-square test. The network was designed in the Pajek 4.09 software (Batagelj & Mrvar, 2003), using the "Kamada-Kawai - separate components" method, in which the vertices (species) with the highest number of connections are attracted to the center of the network.

Results

Literature review

A total of 203 studies met our inclusion criteria. The studies were carried out in 41 countries, with no significant difference in the proportion of studies conducted in temperate (107 studies or 52.7%) and tropical environments (96 studies or 47.3%; $\chi 2 = 0.596$, $p<0.05$; Fig. 2). The number of studies increased considerably from the year 1990 onwards, with the largest number of studies concentrated in the 2010s (61.57%; Fig. 3).

The five most studied crops were also those that had the greatest diversity of pollinator species: watermelon (*Citrullus lanatus*, 60), cucumber (*Cucumis sativus*, 59), bitter gourd (*Momordica charantia*, 51), zucchini/squash/pumpkin (*Cucurbita pepo*, 36) and squash (*Cucurbita moschata*, 31; Table 1). A total of 223 species of pollinators distributed in eight orders (Blattodea, Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mantodea, Odonata and Orthoptera) were registered. The most representative order was Hymenoptera (149 species or 66.81%). Apidae, Halictidae and Megachilidae were the most frequent families of flower visitors (134 species or 89.9%). The second most frequent order was Lepidoptera (58 species or 26%), followed by

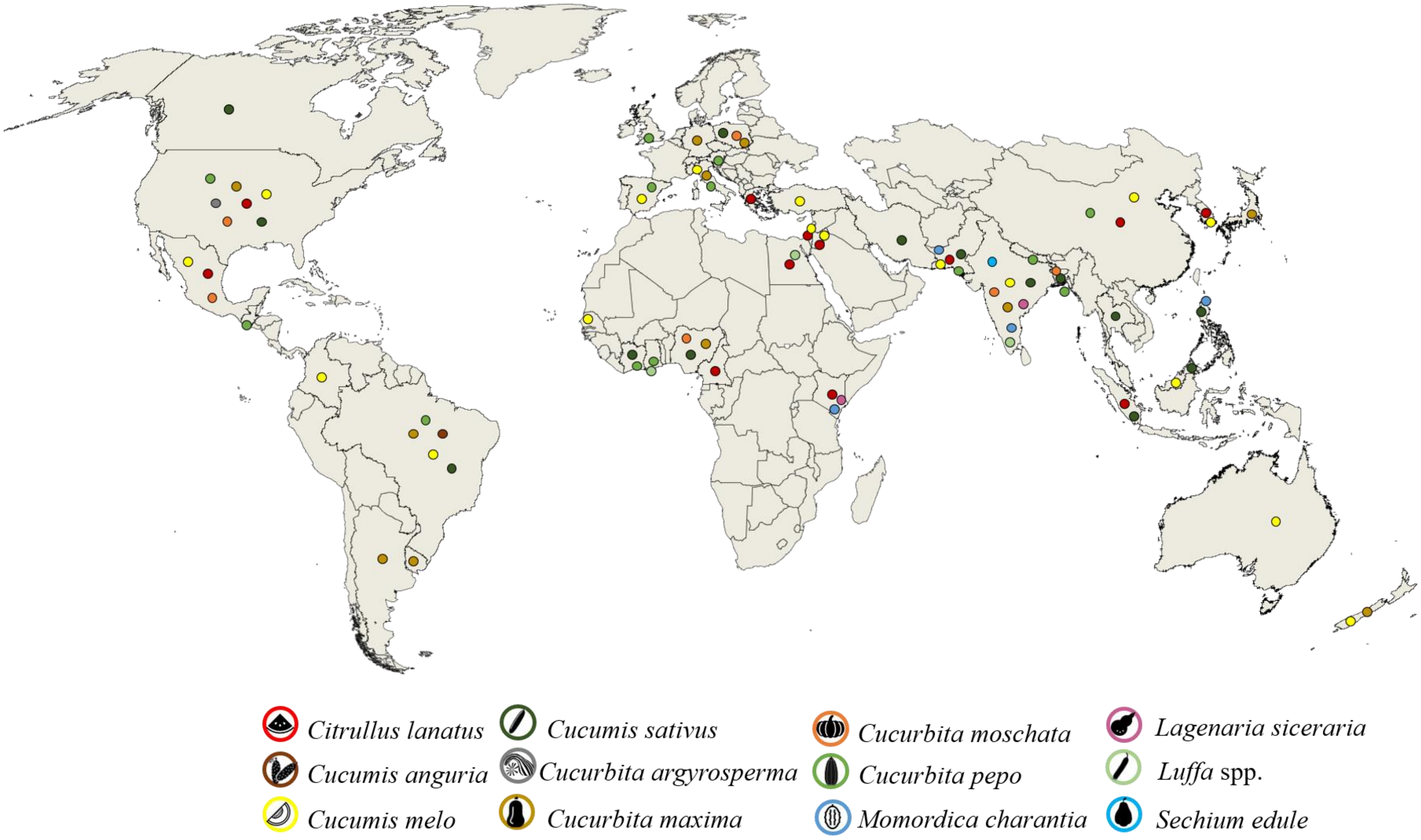

Figure 2. Global distribution of studies on Cucurbitaceae crops pollination.

Table 1. Number (and percentage of the total number of pollinator species recorded) of pollinator species of different taxonomic groups recorded in Cucurbitaceae crops worldwide. Blatto: Blattodea; Coleop: Coleoptera; Lepidop: Lepidoptera; Mantod: Mantodea; Odonat: Odonata; Orthop: Orthoptera; Collet: Colletidae; Eumen: Eumenidae; Formic: Formicidae; Halict: Halictidae; Megach: Megachilidae; Vesp: Vespidae.

Crop	**Blatto**	**Coleop**	**Diptera**	**Hymenoptera**							**Lepidop**	**Mantod**	**Odonat**	**Orthop**
				Apidae	**Collet**	**Eumen**	**Formic**	**Halict**	**Megach**	**Vespid**				
Citrullus lanatus	-	2 (0.89)	4 (1.79)	24 (10.76)	1 (0.44)	2 (0.89)	-	19 (8.52)	1 (0.44)	-	5 (2.24)	-	2 (0.89)	-
Cucumis anguria	-	-	-	1 (0.44)	-	-	-	-	-	-	-	-	-	-
Cucumis melo	-	-	-	18 (8.07)	-	-	-	1 (0.44)	1 (0.44)	-	-	-	-	-
Cucumis sativus	-	1 (0.44)	4 (1.79)	25 (11.21)	-	2 (0.89)	1 (0.44)	5 (2.24)	5 (2.24)	3 (1.34)	13 (5.82)	-	-	-
Cucurbita argyrosperma	-	-	-	1 (0.44)	-	-	-	-	-	-	-	-	-	-
Cucurbita maxima	-	3 (1.34)	1 (0.44)	17 (7.62)	-	-	1 (0.44)	1 (0.44)	-	-	1 (0.44)	-	-	-
Cucurbita moschata	-	-	2 (0.89)	19 (8.52)	-	-	2 (0.89)	5 (2.24)	-	1 (0.44)	2 (0.89)	-	-	-
Cucurbita pepo	-	-	5 (2.24)	28 (12.55)	-	-	-	1 (0.44)	-	-	2 (0.89)	-	-	-
Momordica charantia	-	4 (1.79)	9 (4.03)	15 (6.72)	-	-	1 (0.44)	1 (0.44)	2 (0.89)	1 (0.44)	17 (7.62)	1 (0.44)	-	-
Lagenaria siceraria	-	1 (0.44)	3 (1.34)	6 (2.69)	-	-	1 (0.44)	2 (0.89)	-	-	9 (4.03)	1 (0.44)	-	1 (0.44)
Luffa acutangula	1 (0.44)	3 (1.34)	1 (0.44)	11 (4.93)	-	-	2 (0.89)	-	-	1 (0.44)	9 (4.03)	-	1 (0.44)	1 (0.44)
Luffa aegyptiaca	-	-	-	13 (5.82)	-	-	-	1 (0.44)	1 (0.44)	-	-	-	-	-
Luffa cylindrica	-	-	-	3 (1.34)	-	-	-	-	-	-	-	-	-	-
Sechium edule	-	-	-	4 (1.79)	-	-	-	-	-	-	-	-	-	-

Diptera (29 species or 13%), Coleoptera (14 species or 6.27%), Odonata (three species or 1.34%), Mantodea and Orthoptera (two species each or 0.89%), and Blattodea (one species or 0.44%). The bee *Apis mellifera* was the most recorded species (129 studies or 63.54%), visiting the highest number of plant species (13 species or 92.85%; *Citrullus lanatus*, *Cucumis anguria*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucurbita pepo, Momordica charantia*, *Lagenaria siceraria*, *Luffa acutangula, Luffa aegyptiaca, Luffa cylindrica, Sechium edule*), followed by *Apis dorsata* and *Apis florea* (10 species each), as it is usually a domesticated species.

Based on the number of studies in which the interaction between a plant and a pollinator was recorded, *Bombus impatiens* was the most frequently recorded for watermelon, melon and cucumber crops, *Bombus terrestris* for pumpkin, *Apis dorsata* for cucumber, bitter gourd, and ridge gourd, *Apis cerana* for squash, *Peponapis pruinosa* for *Cucurbita pepo*, *Ceratina smaragdula* for sponge gourd (*Luffa aegyptiaca*), *Xenoglossa kansensis* for squash-neck (*Cucurbita argyrosperma*), *Xylocopa fenestrata* of ridge gourd and the moth *Hippotion celerio* for the bottle gourd species.

Meta-network

We recorded 359 interactions between crops and pollinators (Fig. 4). The meta-network was significantly modular ($Q = 0.55$; $P < 0.001$ for null models), consisting of eight modules containing one or three plant species and 11 to 47 pollinator species. Half of these modules included more than two orders of pollinators, and the frequency functional groups' distribution in the modules was different ($\chi^2 = 111.75$; df = 49; p = 8.33e-07; Fig. 4).

Module 1 consists of squash, sponge gourd (*Luffa cylindrica*) and squash-neck (*Cucurbita argyrosperma*), and 23 pollinators, mainly bees (18 species) and ants (one species). Squash (*Cucurbita moschata*) featured 15 unique species, including bees (13 species), ant (one) and wasp (one). Within this module, squash and sponge gourd (*Luffa cylindrica*) shared *Apis florea*, which had the highest number of links with other plants (ten species) from other modules. Squash-neck shared only one pollinator species (*Xenoglossa kansensis*) with squash and zucchini/squash/pumpkin (module 5).

Module 2 consists of sponge gourd (*Luffa aegyptiaca*) and 11 bee species distributed in the families Apidae (nine species), Halictidae (one species) and Megachilidae (one species).

The genus *Xylocopa* was the most representative (seven species). Five species were exclusive to this module: three of the genus *Xylocopa* and two of *Amegilla*.

Module 3 was composed of bitter gourd and 34 pollinators, from which butterflies (13 species) and flies (eight species) showed highest association level. Twenty-three species were exclusive to this module: butterflies (ten), bees (eight), flies (three) and beetles (two). Syrphidae flies were more representative (seven species) with emphasis on the genus *Eristalinus* (four species). Among the butterflies, the families that stood out were Papilionidae and Pieridae (three species each). Species from this module were shared with five crops (zucchini/squash/pumpkin (*Cucurbita pepo*), cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*), ridge gourd (*Luffa acutangula*) and bottle gourd (*Lagenaria siceraria*).

Module 4 consists of pumpkin and 16 pollinators with beetles (three species) and ants (one species). Nine species are exclusive to this module (six bees and three beetles). *Bombus* had the highest number of species (six).

Module 5 consists of melon, zucchini/squash/pumpkin, and chayote, and with bees (26 species) distributed in the families Apidae (24 species), Halictidae (one species) and Megachilidae (one species). *Bombus* was the most representative genus, with 13 species. Zucchini/squash/pumpkin had 23 pollinator species, melon had 11 species and chayote two species. *Apis cerana* and *Apis dorsata* were shared with the three crops, in addition to having greater links with seven crops from other modules.

Module 6 was composed of cucumber and 34 pollinators, greater associative power with butterflies (nine species) and wasps (three species). Thirty-two species are unique, including bees (19 species), butterflies (nine species), wasps (three species) and ants (one species). Two bee species (*Bombus haemorrhoidalis* and *Heterotrigona itama*) were shared with melon and zucchini/squash/pumpkin.

Module 7 consisting of crop watermelon and 46 unique flower visitors, showed greater association with bees (34 species) and dragonflies (two species). The genus with the greatest diversity was *Lasioglossum* (eight species), followed by *Melissodes* (six species). Finally, Module 8 consists of gherkin, bottle gourd, ridge gourd and composed of 33 pollinators, 24 of which are exclusive, has greater associative power with butterflies (13 species) and with species of Blattodea, Mantodea and Orthoptera (Fig. 5). Bottle gourd with 22 species and ridge gourd with 21 species, both recorded mainly butterflies (nine species). Gherkin presented only one species (*Apis mellifera*), considered the only species shared by the three species present in this module.

Most of the 237 network species (229 species or 96.62%) were classified as peripheral, with few links restricted to the module itself (Fig. 4), of which 93.88% are pollinators (215 species) and 6.11% are crops (14 species; *Citrullus lanatus, Cucumis anguria, Cucumis melo, Cucumis sativus, Cucurbita argyrosperma, Cucurbita maxima, Cucurbita moschata, Cucurbita pepo, Momordica charantia, Lagenaria siceraria, Luffa acutangula, Luffa aegyptiaca, Luffa cylindrica, Sechium edule*). The connector species were the bees *Apis cerana* Fabricius, 1793, *Apis dorsata* Fabricius, 1793, *Ceratina smaragdula* Fabricius, 1787, *Peponapis limitis* Cockerell, 1906, *Tetragonula iridipennis* (Smith, 1854), *Xylocopa aestuans* Linnaeus, 1758, *Xylocopa pubescens* Spinola, 1838, and the ant *Myrmica rubra* Linnaeus, 1758. The only module hub was the bee *Xenoglossa kansensis* Cockerell, 1905, and the network hubs (or super generalists) are the bees *Apis florea* and *Apis mellifera*.

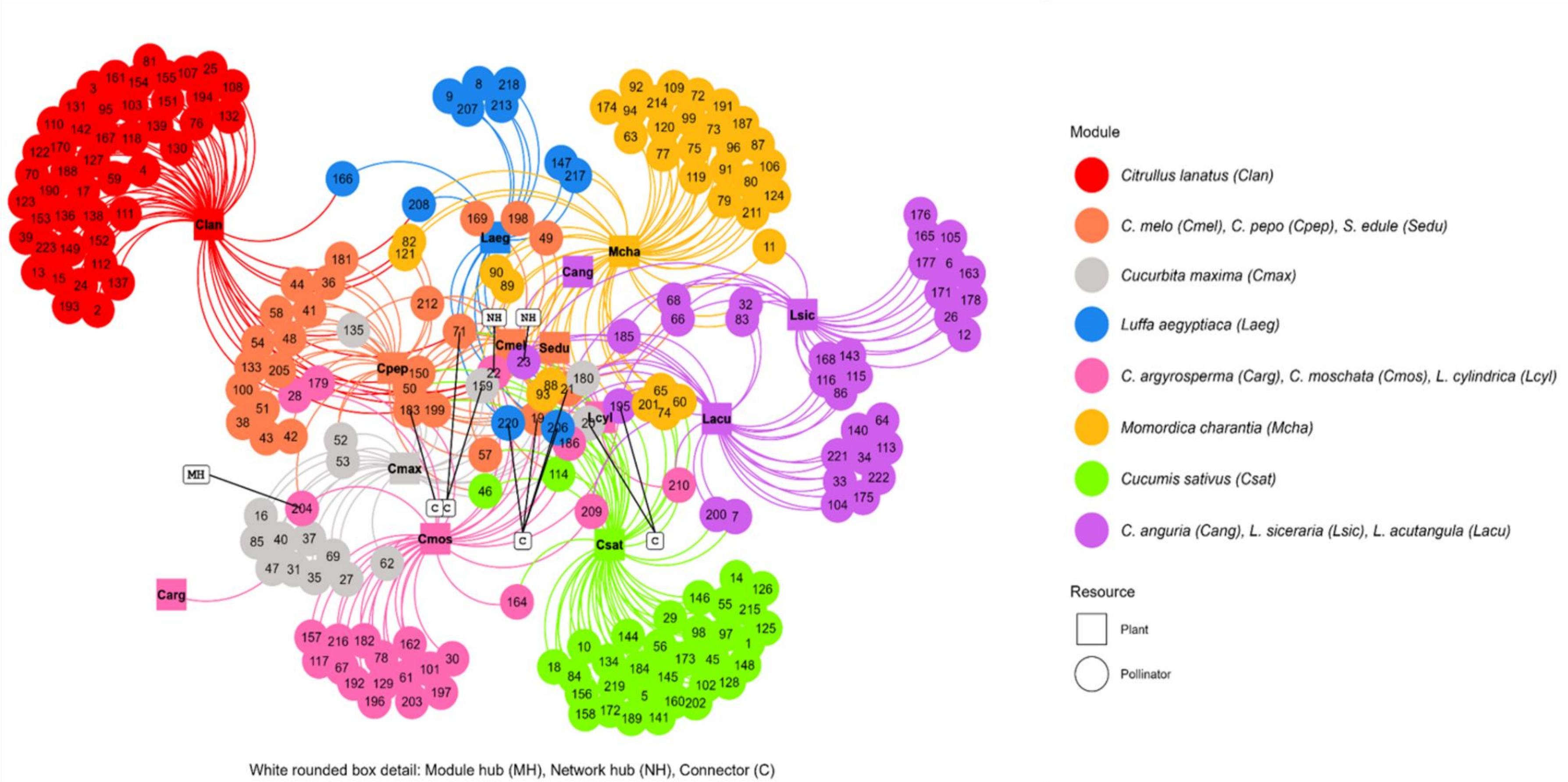

Figure 4. Structure of the modular meta-network representing Cucurbitaceae crops and their pollinators global. Squares: plants; circles: pollinators; Clan: *Citrullus lanatus* (watermelon); Cmel: *Cucumis melo* (melon); Cpep: *Cucurbita pepo* (zucchini); Sedu: *Sechium edule* (chayote); Cmax: *Cucurbita maxima* (pumpkin); Laeg: *Luffa aegyptiaca* (sponge gourd); Carg: *Cucurbita argyrosperma* (gourd-neck); Cmos: *Cucurbita moschata* (squash); Lcyl: *Luffa cylindrica* (sponge gourd); Mcha: *Momordica charantia* (bitter gourd); Csat: *Cucumis sativus* (cucumber); Cang: *Cucumis anguria* (gherkins); Lsic: *Lagenaria siceraria* (bottle gourd); Lacu: *Luffa acutangula* (ridge gourd); MH:

Xenoglossa kansensis; NH: *Apis florea* and *Apis mellifera*; C: *Apis cerana*, *Apis dorsata*, *Ceratina smaragdula*, *Peponapis limitis*, *Tetragonula iridipennis*, *Xylocopa aestuans*, *Xylocopa pubescens* and *Myrmica rubra*. And the floral visitor species (numbers) are defined in the supplementary material.

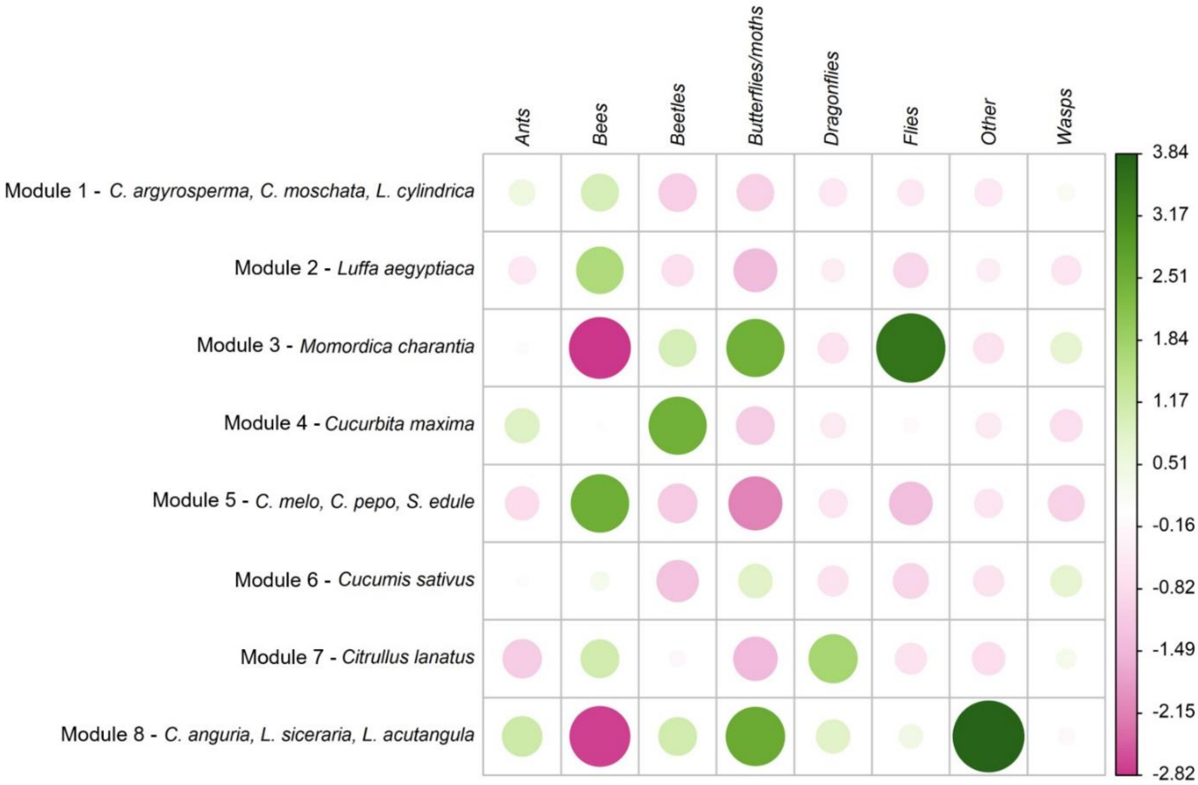

Figure 5. Correlation plot showing the association and disassociation power of the pollinator groups and eight modules detected in the meta-net of interactions between Cucurbitaceae crops and pollinators around the world. Positive values in green indicate attraction/association, and negative values in pink indicate disassociation with the module.

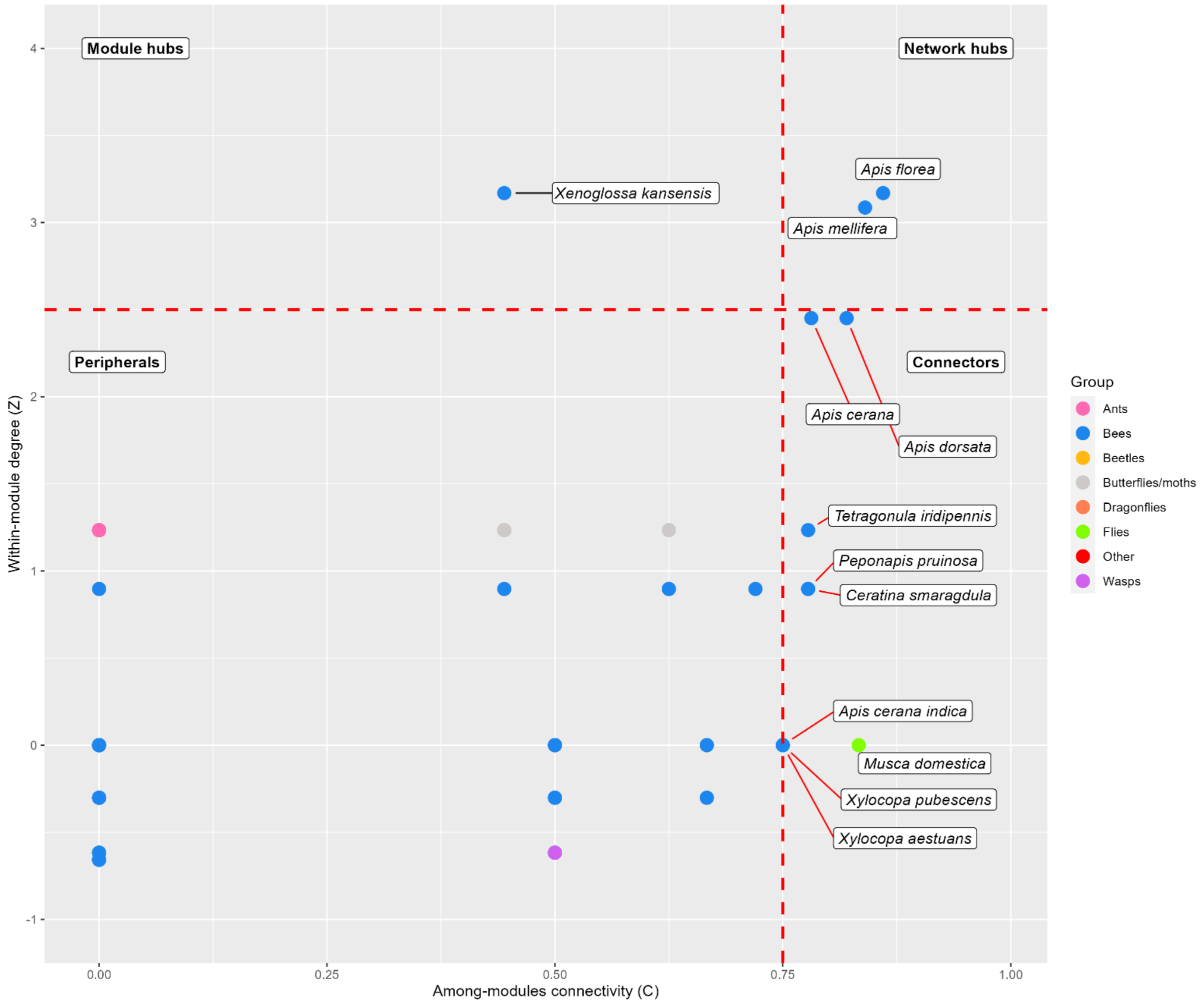

Figure 6. Roles of species in the global meta-network of interactions between Cucurbitaceae crops and pollinators according to their degree within the module (z) and connectivity between modules (c) of each species in each group. Following Dormann and Strauss (2014), we calculated expected c and z values using null models based on the original networks, and used 95% quantiles as critical values.

Discussion

The similar geographic distribution of studies in tropical and temperate regions is a result of the agricultural crop expansion that has occurred in the last 500 years (Brown & Cunningham et al., 2019). Such growth is related to environmental changes (reduction of native habitats, use of pesticides, among others) that, paradoxically, constitute the main causes of the reduction of pollinator populations, with consequent reduction in agricultural production (IPBES, 2016; Dicks et al., 2021). Thus, the increasing number of studies from the 1990s onwards observed here may indicate a concern about the pollinator crisis (Giannini et al., 2015a; Novais et al., 2016), which has been strongly discussed since the 2000s and 2010s, aiming to increase the understanding and mitigation of the global pollinators' decline (Klein et al., 2007; Potts et al., 2010; IPBES, 2016).

The different research intensity observed among crops probably result from their economic importance. Therefore, the greater number of studies with zucchini/squash/pumpkin, watermelon, melon and cucumber may be directly related to their economic value, as they constitute the main cucurbit crops grown worldwide (FAOSTAT, 2021). Consequently, the greater sampling of such crops may explain their greater diversity of flower visitors. Although the bitter gourd (Module 3) is not among the most studied crops, its high diversity of flower visitors may be related to its greater number of flowers available during the flowering period when compared to other cucurbit crops (Subhakar et al., 2011).

The discrepancy in the number of pollinator species among crops may indicate that the global decline of pollinators may not affect all crops equally. Crops that are pollinated by a greater number of species have, in principle, greater resilience when compared to crops pollinated by a reduced number of species (IPBES, 2016), as is the case of squash-neck and sponge gourd (*Luffa cylindrica*), chayote and gherkin. The growing of cucurbit plants outside their original range may also shifts the distribution limits of their associated pollinators in rare cases (López-Uribe et al., 2016), in addition to the landscape and local habitat scales (nesting resources, climate, among others) It is important to note, however, that such an assumption is more complex than it seems, as it is necessary to understand whether pollinators perform effectively (Nicholson et al., 2019; Rollin & Garibaldi, 2019; Kendall et al., 2021). In addition, high visitation rates can damage flowers, resulting in detrimental effects on fruit set (Young, 1988; Young & Young 1992; Morris et al., 2010; Sáez et al., 2014).

The high diversity of pollinators recorded here was grouped into modules, similarly to what was observed in studies involving a meta-network approach to pollination in communities of grasses (Danieli-Silva et al., 2012), of tropical species from the Peruvian Andes (Watts et al., 2016), urban environments (Nascimento et al., 2020) and legumes of economic importance (Paulino et al., 2021). Patterns of plant-pollinator interaction in a meta-network can be explained by several factors (Araujo et al., 2018). The presence of plants and pollinators in the modules can be influenced by functional traits of plants and pollinators and the spatial distribution of species. In the case of the plants in our network, some functional traits may explain modules formation, but in a general matter this is not clear. The factors that determine the spatial distribution include climatic conditions and tradition/vocation of the region in producing that group of crops.

The predominance of pollinators among the peripherals indicate that they have few interactions mostly within their module (Olesen et al., 2007; Martín González et al., 2012). Regarding general features of the connector species, most are small to medium-sized bees. *Ceratina smaragdula* is considered a potentially important pollinator of legumes and cucurbits (Ali et al., 2016). The social stingless bee *Tetragonula iridipennis* has high potential to efficiently pollinate various agricultural crops, as it can collect floral resources from a broad range of flower sizes and anthers' structures (Makkar et al., 2016; Bisui et al., 2021). It promotes a significant increase in the yield of cucumber production, both in terms of quantitative parameters (number of fruits per plant) and qualitative parameters (length, circumference, and weight of fruits; Kishan et al., 2017). *Xylocopa pubescens* is a large, facultatively social and generalist carpenter bee (Hogendoorn & Velthuis, 1995).

The predominance of pollination by bees of the Apidae family occurred mainly by *Apis*, *Bombus* and *Xylocopa* species, considered the main pollinators of Cucurbitaceae (Mensah & Kudom, 2011; Campbell et al., 2018). They are generalist, exploring flowers with different morphologies and resources, thus visiting a great diversity of plants (Russell et al., 2017; Layek et al., 2020).

Apis bees are considered a less efficient species in pollinating cucurbits than *Peponapis* ones, as they transfer smaller pollen loads in single visits to flowers (Delgado-Carrillo et al., 2018). Additionally, *Apis* bees tend to visit mainly female flowers, whose nectar volume and concentration are higher than that of male flowers (Artz & Nault, 2011; McGrady et al., 2020). On the other hand, pollination by *A. mellifera* visits last three times longer, during all seasons (Delgado-Carrillo et al., 2018), and visiting activity extends to the night for *Apis dorsata* (Balachandran et al., 2017). Honeybees are thus considered an important pollinator of Cucurbitaceae when there are no native species in crops.

Although *A. mellifera* was recorded in almost all crops, it was only considered the main pollinator species of gherkin, probably due to the lack of records with other pollinators. Even though *A. mellifera* was considered a network hub, it is important to stress that the high abundance of these bees can interfere with many flower-pollinator interactions, either directly affecting flower performance or reducing the pollination efficiency of other floral visitors, with negative consequences for pollination and crop yield (Aizen et al., 2020; Garibaldi et al., 2021). *Apis mellifera* is known to negatively impact the reproductive success of plants pollinated by native pollinators, causing a reduction in the diversity of these animals, as well as decreasing the number of interactions in pollinator networks (Valido et al., 2019). In addition, non-native bees can compete with native bees for nesting sites or floral resources (Russo et al., 2021). Therefore, we must promote the use of native bees, as they provide essential pollination services in natural and managed ecosystems, increasing the yield of various crops (Appenfeller et al., 2020; Layek et al., 2021). Bee species such as *Peponapis* are great examples, as they are specialists in *Cucurbita* pollen, visiting pumpkin flowers before other species, transferring four times more viable pollen than other pollinating species, such as *A. mellifera* (Delgado-Carrillo et al., 2018). Furthermore, *Peponapis* bees are obligatory dependent on resources provided by flowers of the genus *Cucurbita* for larval and adult nutrition, and their life cycle is synchronized with the natural flowering period of pumpkin crops undergoing a hibernation period as a pre-pupa in the soil (Hurd et al., 1971; Delgado-Carrillo et al., 2017).

The generalist foraging strategies of *Apis* species are resulted from selective pressures favoring the accommodation of nutritional needs of their colonies inhabited by many individuals (Koppler et al., 2007), being able to transport varied amounts of food. The only two network hubs in this study are included in *Apis* genus (*A. florea* and *A. mellifera*) and constitute important pollinators for agricultural production worldwide, since they have generalist behavior, being able to efficiently pollinate a wide range of floral types (El Shafie et al., 2002; Garibaldi et al., 2014; Rajan & Reddy, 2019; Vidhya et al., 2019). *Apis florea*, for example, exhibits this type of behavior due to its ability to be highly migratory (Ruttner et al., 1995). However, it is a bee with difficult domestication and very sensitive to temperature fluctuations (Rajan & Reddy, 2019). It occurs in semi-arid to tropical environments in various regions of Asia and Africa (Sihag, 2021) and tends to compete with *Apis mellifera* during foraging, possibly stealing its colonies (Koeniger, 1976; Chahal et al., 1986). *Apis mellifera* is considered as a super generalist because it is adapted to different climatic conditions and habitats, not suffering damage in degraded environments (Kleinert & Giannini, 2012; Giannini et al., 2015b), in addition to being less sensitive to the perception of floral volatiles, which make them less specific in choosing plant species (Burger et al., 2013; Liu et al., 2022).

B*ombus* species have a relatively large body and dense hairs, capable of depositing more pollen grains on a stigma per single visit than other bees (Artz & Nault, 2011) and contact the stigmas significantly more frequently than *A. mellifera* or *Peponapis pruinosa*. In addition, they exhibit a rapid extraction of nectar and quick flights between flowers, making them efficient pollinators of *Cucurbita* (Lobo & Mendez, 2021). Those bees effectively forage a wide range of flowers due to their behavioral flexibility, being able to vibrate during pollen collection (Russel et al., 2017). An efficient pollen collection favors obtaining high amounts of protein resulting in larger offspring (Tasei & Aupinel, 2008). *Bombus impatiens* is an important pollinator of cucurbits, where the highest yield occurs with high visitation rates (Petersen et al., 2013), particularly for watermelon and pumpkin flowers (Campbell et al., 2018).

Carpenter bees (*Xylocopa*) are very important pollinators for several agricultural crops, including cucurbit species (Ali et al., 2016), because of their large size, which improve pollination when compared to other smaller bees (Mensah & Kudom, 2011). They also move quickly from flower to flower in large areas (Mensah & Kudom, 2011) and forage in the early morning, when flowers have large amounts of resources (Azo'o et al., 2020). Among the advantages of *Xylocopa* bees as potential pollinators, foraging tolerance at high temperatures, activity in long seasons, foraging in a wide variety of crops and activity under low lighting levels stand out (Keasar, 2010).

The bee *Xenoglossa kansensis* is considered one of the main pollinators of *Cucurbita* species (Hurd & Linsley, 1964; Hurd et al., 1971), as it visits exclusively flowers of zucchini/squash/pumpkin, gourd-neck, pumpkin, and squash, contributing significantly to its production. The species is restricted to North America, especially North Florida, and has its flight period in spring (Wille, 1985; Hall, 2010).

Bees of the genus *Peponapis* are oligolectic on cucurbits. Normally, mating occurs in flowers and serve as shelter for male bees (Hurd et al., 1971; Willis & Kevin, 1995). The specialized squash bee, *Peponapis pruinosa*, is a soil-nesting pollinator of cucurbit crops, mainly of the genus *Cucurbita* (Mathewson, 1968; Hurd et al., 1974; Julier & Roulston, 2009). Although these bees are negatively affected by frequent cultivation when compared to uncultivated areas, they can survive as disturbed agricultural fields provide essential floral resources for nesting, contributing to their persistence (Ullmann et al., 2016). They visit the squash flowers more quickly than *A. mellifera*, making it unnecessary to install honey bee colonies in crops where the squash bee population is large (Tepedino, 1981). *Peponapis limitis* is considered a key species in the cultivation of *Cucurbita mochata* due to its high frequency of visits and efficiency in the removal and deposition of pollen, with female bees capable of depositing four times more pollen in the pistillate flowers than in a single visit of *Apis mellifera* (Canto-Aguilar et al., 2000).

The associative power of a functional group in a module refers to the group of pollinators that most strongly differentiates the module when compared to the others. Therefore, although bees are present in different proportions in all modules, modules 1 (squash, sponge gourd – *Luffa cylindrica* and squash-neck), 2 (sponge gourd – *Luffa aegyptiaca*) and 5 (melon, zucchini/squash/pumpkin, and chayote) showed the highest power of association with these insects. The floral characteristics of cucurbits also tend to attract bees, due to their large and showy flowers, with petals that vary in color from bright yellow to white (Richards, 1986; Nepi & Pacini, 1993), and a mild odor that is dispersed by long distance mainly due to solar radiation (Agbagwa et al., 2007). Additionally, the flowers have a high availability of pollen and nectar with high sugar concentration throughout the flowering period (Vidal et al., 2010).

Module 3 (bitter gourd) was associated with dipterans, and Syrphidae family was the most predominant. Female flowers of this crop do not produce nectar; thus, the only floral reward comes from male flowers. Those dipterans are attracted by yellow actinomorphic flowers (Sajjad & Saeed, 2010) with abundant floral resources (pollen and nectar), that use pollen for egg formation and nectar for self-nutrition (Moquet et al., 2018). Migratory species gain prominence as pollinators because they can transport pollen grains over long distances, promoting the interaction of isolated plants (Doyle et al., 2020). It is interesting to note that the bitter gourd has similar proportion of male and female flowers when compared to cucumber and sponges and similar size, thus functional traits may be related to the predominance of hoverflies.

The strong association of modules 3 (bitter gourd), 6 (cucumber) and 8 (gherkin, bottle gourd, ridge gourd) with lepidopterans can be justified by the high number of studies on these crops that recorded lepidopterans when compared to the number of studies that recorded other functional groups of pollinators. Additionally, floral attributes of those crops may explain the presence of Lepidopterans. Anthesis can be diurnal or even last until the next day (bottle gourd and ridge gourd), allowing availability of floral rewards (pollen and nectar) for long periods, favoring nocturnal pollinators. The crops of this module have yellow and white corollas, which attract lepidopterans (Yurtsever et al., 2010). Additionally, the flat structure of the flowers of most plants in this module allows lepidopterans to land on flowers more easily during floral resources' collection (Goulson, 1999). Bitter gourd, cucumber and gherkin have flowers with diurnal anthesis, abundant nectar, which is the main source of nutrients for lepidopterans, and a mild odor, which tend to attract these pollinators (Lenzi et al., 2005; Borges et al., 2016; Patil & Jagdale, 2021).

The greater power of association of the order Coleoptera with module 4 (pumpkin) can be explained by the fact that it is the only crop that had beetles as the second most representative group of pollinators after bees. Furthermore, the composition of the floral odor of pumpkin flowers acts as a potential source of attraction for beetles (Andersen, 1987; Andrews et al., 2007). Even the species of *Cucurbita* having a high concentration of cucurbitacin, which act in the defense of the plant against the presence of insects, some species of beetles prefer their flowers, due to the emission of volatile sesquiterpenoids, which are associated with the attraction of animals, and may help in the interaction with pollinators (Theis et al., 2014). Although beetles are considered less specialized pollinators than bees, due to their random movements within the flower and their flights over small distances between flowers of the same plant (Lloyd & Schoen, 1992; Ashworth & Galetto, 2001), they can carry out pollination. *Aulacophora femoralis*, for example, has high pollination efficiency after visiting pumpkin plants (*Cucurbita maxima*, Kumar et al., 2012).

Module 7 (watermelon) exhibited greater association with the order Odonata, which includes *Hemianax ephippiger* and *Ischnura senegalensis*. Odonata has been reported in only one study, and we doubt that dragonflies act as pollinators, however this study was included for completeness. It is important to stress that a high abundance of dragonflies in plantations may result in a reduction of several groups of pollinators, as dragonflies are insect predators (Knight et al., 2005; Tiitsaar et al., 2013; May, 2019). Their presence tends to mainly reduce the number of bee species, consequently increasing the magnitude of the pollen limitation, and therefore decreasing seed set (Knight et al., 2005).

The number of studies recording the interaction of a crop with a specific group of pollinators is an important factor determining the modules of a network (Heilmann-Clausen et al., 2016; Araujo et al., 2018). However, we faced some limitations in the selection of data from the literature, such as the exclusion of studies written in languages other than English, as well as studies that did not record pollinators at the species level. Even in the studies that were included, there is still a likelihood that not all flower visitors were recorded. Although these limitations were found, this study allows for a broad view of the composition of the pollinating fauna of Cucurbitaceae species. In general, cucurbit species have a great diversity of pollinators, making it possible to observe which species are specific or shared with other plants. There is a difference in the number of pollinators registered for each region, with the United States having the highest number of species, as well as the highest number of studies. As implications for pollinator conservation, we highlight that plant-pollinator interaction networks show that Cucurbitaceae crops have many specific species, but *Apis mellifera* bees dominate interactions in different countries. Therefore, to improve pollinator conservation, the management of native species is recommended.

Conclusions

Although cucurbits flowers are, in a general matter, similar in color and shape, they are pollinated by a high diversity of insects. Probably this occurs because of variations on other flower attributes. The global pollination meta-network of Cucurbitaceae crops was highly modular and bees were structurally the most important pollinators. However, non-bee insects such as butterflies, flies, beetles, ants, and wasps also played a role in the network. Modules formation may be related to functional traits of plants and pollinators, geographical distribution of studies seems to have an influence. Some biases of the study are the limitation of the literature selection and the exclusive use of studies that bring the identification of pollinators until species level. Research intensity on crops certainly results from their economic importance. Although there is an increasing number of studies on the interaction of pollinators and Cucurbitaceae crops, no studies have compiled these data. Our study will serve as a source of data for the development of management and conservation practices for the species since it shows important native pollinator species *Peponapis pruinosa* and *Xenoglossa* can help to mitigate the global pollinator crisis. Our data shows the importance of native bees, which are the good pollinators and an alternative to exotic species. It is important to maintain nesting resources for ground-nesting bees, often neglected in conservation. Gherkin, squash, chayote, and sponge gourd (*Luffa aegyptiaca*) need further pollination studies. Investigations on the differential efficiency of native, manageable pollinators are necessary.

Acknowledgements

We thank the Programa de Pós-Graduação em Biodiversidade of the Universidade Federal Rural de Pernambuco and the Universidade Federal do Agreste de Pernambuco for the institutional support. The authors do not have conflicts of interest.

Competing Interests

Authors have no competing interests.

Funding

ICSM received financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Financing Code 001), and CCC from Conselho Nacional de Desenvolvimento Científico e Tecnológico (research grant: 428303/2018-8, fellowship: 312372/2021-3).

Author contribution

ICSM carried out the research and conducted data collection.

KRS conducted statistical analyses.

CCC coordinated the study and obtained funding.

All authors wrote and approved the manuscript.

Data Availability Statement

All data used in this study will be available at Figshare: Castro (2023). Cucurbitaceae crops' pollinators around the world. DOI: 10.6084/m9.figshare.22806854

References

Agbagwa, I. O., Ndukwu, B. C. & Mensah, S. I. (2007). Floral biology, breeding system, and pollination ecology of *Cucurbita moschata* (Duch. ex Lam) Duch. ex Poir. varieties (Cucurbitaceae) from parts of the Niger Delta, Nigeria. *Turkish Journal of Botany*, *31*, 451-458.

Aizen, M. A., Aguiar, S., Biesmeijer, J. C., Garibaldi, L. A., Inouye, D. W., Jung, C., Martins, D. J., Medel, R., Morales, C. L., Ngo, H., Pauw, A., Paxton, R. J., Sáez, A. & Seymour, C. L. (2019). Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. *Global Change Biology*, *25*, 3516-3527. DOI: 10.1111/gcb.14736

Aizen, M. A., M. P. Arbetman, N. P. Chacoff, V. R. Chalcoff, P. Feinsinger, L. A. Garibaldi, L. D. Harder, C. L. Morales, A. Sáez, & A. J. Vanbergen. (2020). Invasive bees and their impact on agriculture. *Advances in Ecological Research*, *63*, 49-92. DOI: 10.1016/bs.aecr.2020.08.001

Ali, H., Shebl, M., Alqarni, A. S., Owayss, A. A. & Ansari, M. J. (2016). Nesting biology of two species of the large carpenter bees *Xylocopa pubescens* and *Xylocopa fenestrata* (Hymenoptera: Apidae) in north-western Pakistan. *Oriental Insects*, *51*, 1-12. DOI: 10.1080/00305316.2016.1272500

Amadou, I. & Bako, A. T. (2018). Perception of *Lagenaria siceraria* Fruits on Their Nutritive, Therapeutic and Organoleptic Values. *American Journal of Food, Nutrition and Health*, *3*, 26-30.

Andersen, J. F. (1987). Composition of the floral odor of *Cucurbita maxima* Duchesne (Cucurbitaceae). *Journal of Agricultural and Food Chemistry*, *35*, 60-62.

Andrews, E. S., Theis, N. & Adler, L. S. (2007). Pollinator and herbivore attraction to *Cucurbita* floral volatiles. *Journal of Chemical Ecology*, *33*, 1682-1691. DOI: 10.1007/s10886-007-9337-7

Appenfeller, L. R., Lloyd, S. & Szendrei, Z. (2020) Citizen science improves our understanding of the impact of soil management on wild pollinator abundance in agroecosystems. *PLoS ONE*, *15*, e0230007. DOI: 10.1371/journal. pone.0230007

Araujo, A. C., González, A. M. M., Sandel, B., Maruyama, P. K., Fischer, E., Araújo, F. P., Coelho, A. G., Faria, R. R., Kohler, G., Las-Casas, F. M. G., Lopes, A. V., Machado, A. O., Machado, C. G., Machado, I. C., McGuire, J. A., Moura, A. C., Oliveira, G. M., Oliveira, P. E., Rocca, M. A., Rodrigues, L. C., Rodrigues, M., Rui, A. M., Sazima, I., Varassin, I. G., Wang, Z., Dalsgaard, B. & Svenning, J. C. (2018). Spatial distance and climate determine modularity in a cross-biomes plant–hummingbird interaction network in Brazil. *Journal of Biogeography*, *45*, 1846-1858. DOI: 10.1111/jbi.13367

Artz, D. R. & Nault, B. A. (2011). Performance of *Apis mellifera*, *Bombus impatiens*, and *Peponapis pruinosa* (Hymenoptera: Apidae) as Pollinators of Pumpkin. *Journal of Economic Entomology*, *104*, 1153-1161. DOI: 10.1603/ec10431

Ashworth, L. & Galetto, L. (2001). Pollinators and Reproductive Success of the Wild Cucurbit *Cucurbita maxima* ssp. *andreana* (Cucurbitaceae). *Plant Biology*, *3*, 398-404. DOI: 10.1055/s-2001-16451

Azo'o, E. M., Bissou, W. B. & Tchuenguem, F. F. N. (2020). Comparing the foraging behaviour and pollination efficiency of *Apis mellifera* with *Xylocopa olivacea* (Apidae: Hymenoptera) on *Citrullus lanatus* flowers. *Journal of Applied Horticulture*, *22*, 18-23. DOI: 10.37855/jah.2020.v22i01.04

Bomfim, I. G. A., Cruz, D. O., Freitas, B. M., Aragão, F. A. S. (2013). Polinização em melancia com e sem semente / – Fortaleza: Embrapa Agroindústria Tropical, 2013. 53 p.– (Documentos / Embrapa Agroindústria Tropical).

Balachandran, C., Chandran, M. D. S., Vinay, S., Shrikant, N. & Ranachandra, T. V. (2017). Pollinator diversity and foraging dynamics on monsoon crop of cucurbits in a traditional landscape of south indian west coast. *Biotropia The Southeast Asian Journal of Tropical Biology*, *24*, 16-27. DOI: 10.11598/btb.2017.24.1.480

Barghamdi, B., Ghorat, F., Asadollahi, K., Sayehmiri, K., Peyghambari, R. & Abangah, G. (2016). Therapeutic effects of *Citrullus colocynthis* fruit in patients with type II diabetes: a clinical trial study. *Journal of Pharmacy & Bioallied Sciences*, *8*, 130-134. DOI: 10.4103/0975-7406.171702

Bascompte J, Jordano P (2007) Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, 38, 567-593. DOI: 10.1146/annurev.ecolsys.38.091206.095818

Batagelj, V. & Mrvar, A. (2003). Pajek: Analysis and visualization of large networks. Preprint series, 41, 1-28.

Beckett, S. J. (2016). Improved Community detection in weighted bipartite networks. *Royal Society Open Science*, *3*, 1-18. DOI: 10.1098/rsos.140536

Bisui, S., Layek, U., & Karmakar, P. (2021). Determination of Nectar Resources through Body Surface Pollen Analysis: A Study with the Stingless Bee *Tetragonula iridipennis* Smith (Apidae: Meliponini) in West Bengal, India. *Sociobiology*, *68*, e6173. DOI: 10.13102/sociobiology.v68i3.6173

Blüthgen, N., Menzel, F. & Blüthgen, N. (2006). Measuring specialization in species interaction networks. *Ecology*, *6*, 1-12. DOI:10.1186/1472-6785-6-9

Borges, R. M., Somnathan, H., Kelber, A. (2016). Patterns and processes in nocturnal and crepuscular pollination services. *The Quarterly Review of Biology*, *91*, 389-418. DOI: 10.1086/689481

Brown, J. & Cunningham, S. A. (2019). Global-scale drivers of crop visitor diversity and the historical development of agriculture. *Proceedings of the Royal Society B: Biological Sciences*, 286 (1915), 20192096. DOI: 10.1098/rspb.2019.2096

Burger, H., Ayasse, M., Dötterl, S., Kreissl, S. & Galizia, C. G. (2013). Perception of floral volatiles involved in host-plant finding behaviour: comparison of a bee specialist and generalista. *Journal of Comparative Physiology A*, *199*, 751-761.

Cagua, E. F., Wootton, K. L. & Stouffer, D. B. (2019). Keystoneness, centrality, and the structural controllability of ecological networks. *Journal of Ecology*, 107 (4), 1779-1790. DOI: 10.1111/1365-2745.13147

Campbell, J. W., Daniels, J. C. & Ellis, J. D. (2018). Fruit Set and Single Visit Stigma Pollen Deposition by Managed Bumble Bees and Wild Bees in *Citrullus lanatus* (Cucurbitales: Cucurbitaceae). *Journal of Economic Entomology*, *111*, 989-992. DOI: 10.1093/jee/toy008

Canto-Aguilar, M. L. & Parra-Tabla, V. (2000). Importance of conserving alternative pollinators: assessing the pollination efficiency of the squash bee, *Peponapis limitaris* in *Cucurbita moschata* (Cucurbitaceae). *Journal of Insect Conservation*, 4 (3): 201-208. DOI:10.1023/A:1009685422587.

Carneiro Neto, T. F. S., Silva, G. B. S.; Almeida Neto, S. V. De; Rodrigues, R. M. P.; Feitosa, M. S., Duarte, P. M., Silva, N. C., Siqueira, K. M. M. (2018). Floração e Biologia Floral do Maxixeiro. *Revista Ouricuri*, *8*, 057-068, DOI: 10.29327/ouricuri.v8.i1.a6.

Chahal, B. S., Brar, H. S., Gatoria, G. S. & Jhajj, H. S. (1986). Aggressive behavior of *Apis florea* towards *Apis mellifera* in hive robbing and in foraging. *Journal of Apicultural Research*, *25*, 134-138. DOI: 10.1080/00218839.1986.11100706

Chomicki, G., Schaefer, H. & Renner, S. S. (2020). Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. *New Phytologist*, *226*, 1240-1255. DOI: 10.1111/nph.16015

Danieli-Silva, A., Souza, J. M. T., Donatti, A. J., Campos, R. P., Vicente-Silva, J, Freitas, L. & Varassin, I. G (2012). Do pollination syndromes cause modularity and predict interactions in a pollination network in tropical high-altitude grasslands? *Oikos*, *121*, 35-43. DOI: 10.1111/j.1600-0706.2011.19089.x

Delgado-Carrillo, O., Lopezaraiza-Mikel, M., Ashworth, L., Aguilar, R., Lobo, J. A. & Quesada, M. (2017). A scientific note on the first record of nesting sites of *Peponapis crassidentata* (Hymenoptera: Apidae). *Apidologie*, *48*, 644-647.

Delgado-Carrillo, O., Martén-Rodríguez, S., Ashworth, L., Aguilar, R., Lopezaraiza-Mikel, M. & Quesada, M. (2018). Temporal variation in pollination services to *Cucurbita moschata* is determined by bee gender and diversity. *Ecosphere*, *9*, e02506. DOI: 10.1002/ecs2.2506

Dicks, L.V., Breeze, T. D., Ngo, H. T., Senapathi, D., An, J., Aizen, M.A., Basu, P., Buchori, D., Galetto, L., Garibaldi, L. A., Gemmill-Herren, B., Howlett, B. G., Imperatriz-Fonseca, V. L., Johnson, S. D., Kovács-Hostyánszki, A., Kwon, Y. J., Lattorff, H. M. G., Lungharwo, T., Seymour, C. L., Vanbergen, A. J. & Potts, S. G. (2021). A global-scale expert assessment of drivers and risks associated with pollinator decline. *Nature Ecology & Evolution*, *5*, 1453-1461.

Donoso, S. & Murúa, M. (2021). Floral Patches and Their Impact on Pollinator Attraction and Yield Production on *Cucurbita maxima* Var. Paine in Central Chile. *Diversity*, *13*, 608. DOI: 10.3390/d13120608

Dormann, C. F., Frund, J. & Gruber, B. (2008). Introducing the bipartite package: analysing ecological networks. - *R News*, *8*, 8-11.

Dormann, C. F. & Strauss, R. (2014). A method for detecting modules in quantitative bipartite networks. *Methods in Ecology and Evolution, 5*, 90–9. DOI: 10.1111/2041-210X.1213

Doyle, T., Hawkes, W. L. S., Massy, R., Powney, G. D., Menz, M. H. M. & Wotton, K. R. (2020). Pollination by hoverflies in the Anthropocene. *Proceedings of the Royal Society B: Biological Sciences*, 287 (1927), 20200508. DOI: 10.1098/rspb.2020.0508

El Shafie, H. A. F., Mogga, J. B. B. & Basedow, T. (2002). Studies on the possible competition for pollen between the honey bee, *A. mellifera* sudanensis, and the imported dwarf honey bee *A. florea* (Hym., Apidae) in North-Khartoum (Sudan). *Journal of Applied Entomology*, *126*, 557-562. DOI: 10.1046/j.1439-0418.2002.00711.x

Eilers, E. J., Kremen, C., Smith Greenleaf, S., Garber, A. K. & Klein, A. M. (2011). Contribution of Pollinator-Mediated Crops to Nutrients in the Human Food Supply. *PLoS One*, *6*, e21363. DOI: 10.1371/journal.pone.0021363

Ekeke, C., Ogazie, C. A., Agbagwa, I. O. (2018). Breeding biology and effect of pollinators on the fruit characteristics of cucumber (*Cucumis sativus* L.), Cucurbitaceae. *Nigerian Journal of Botany*, *31*, 325-344.

Emer, C., Galetti, M., Pizo, M. A., Guimarães Jr., P. R., Moraes, S., Piratelli, A. & Jordano, P. (2018). Seed-dispersal interactions in fragmented landscapes – a metanetwork approach. *Ecology Letters*, *21*, 484-493. DOI: 10.1111/ele.12909

Enríquez, E., Ayala, R., Gonzalez, V. H., Núñez-Farfán, J. (2015). Alpha and beta diversity of bees and their pollination role on *Cucurbita pepo* L. (Cucurbitaceae) in the Guatemalan cloud forest. *The Pan-Pacific Entomologist*, *91*, 211-222. DOI: 10.3956/2015-91.3.211

FAOSTAT (2022). Food and Agriculture Organization of the United Nations. Disponível em: <http://faostat.fao.org/ >. Acesso em: 20 de dez. 2022.

Fijen, T. P. M., Scheper, J. A., Boom, T. M., Janssen, N., Raemakers, I. & Kleijn, D. (2018). Insect pollination is at least as important for marketable crop yield as plant quality in a seed crop. *Ecology Letters*, *21*, 1704-1713. DOI: 10.1111/ele.13150

Free, J. B. (1993) Insect pollination of crops. 2ª ed. Academic Press Londres, 684p.

Fründ, J., McCann, K. S. & Williams, N. M. (2016). Sampling bias is a challenge for quantifying specialization and network structure: lessons from a quantitative niche model. *Oikos*, *125*, 502-513. DOI: 10.1111/oik.02256

Garibaldi, L. A., Pérez-Méndez, N., Cordeiro, G. D., Hughes, A., Orr, M., Alves-dos-Santos, I., Freitas, B. M., Oliveira, F. F., LeBuhn, G., Bartomeus, I., Aizen, M. A., Andrade, P. B., Blochtein, B. et al. (2021). Negative impacts of dominance on bee communities: Does the influence of invasive honey bees differ from native bees? *Ecology*, *102*, e03526. DOI: 10.1002/ecy.3526

Garibaldi, L. A., Carvalheiro, L. G., Leonhardt, S. D., Aizen, M. A., Blaauw, B. R., Isaacs, R., Kuhlmann, M., Kleijn, D., Klein, A. M., Kremen, C., Morandin, L., Scheper, J. & Winfree, R. (2014). From research to action: enhancing crop yield through wild pollinators. *Frontiers in Ecology and the Environment*, *12*, 439-447. DOI: 10.1890/130330

Giannini, T. C., Cordeiro, G. D., Freitas, B. M., Saraiva, A. M. & Imperatriz-Fonseca, V. L. (2015a). The dependence of crops for pollinators and the economic value of pollination in Brazil. *Journal of Economic Entomology*, *108*, 849-857. DOI: 10.1093/jee/tov093

Giannini, T. C., Garibaldi, L. A., Acosta, A. L., Silva, J. S., Maia, K. P., Saraiva, A. M., Guimarães Jr., P. R. & Kleinert, A. M. P. (2015b). Native and non-native supergeneralist bee species have different effects on plant-bee networks. *PLoS ONE*, *10*, 1-13. DOI: 10.1371/journal.pone.0137198

Gómez, S. R., Ornosa, C., Selfa, J., Guara, M. & Polidori, C. (2016). Small sweat bees (Hymenoptera: Halictidae) as potential major pollinators of melon (*Cucumis melo*) in the Mediterranean. *Entomological Science*, *19*, 55-66. DOI: 10.1111/ens.12168

González, A. M. M., Dalsgaard, B. & Olesen, J. M. (2010). Centrality measures and the importance of generalist species in pollination networks. *Ecological Complexity*, *7*, 36-43. DOI: 10.1016/j.ecocom.2009.03.008

Goulson, D. (1999). Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. *Perspectives in Plant Ecology, Evolution and Systematics*, *2*, 185-209. DOI: 10.1078/1433-8319-00070

Hall, H. G. (2010). The Squash Bee *Xenoglossa kansensis* Cockerell (Hymenoptera: Apidae) Found in Organic Farms in Northern Florida. *Journal of the Kansas Entomological Society*, *83,* 84-88. DOI: 10.2317/0022-8567-83.1.84

Heilmann-Clausen J. (2016). Citizen science data reveal ecological, historical and evolutionary factors shaping interactions between woody hosts and wood-inhabiting fungi. *New Phytologist*, *212*, 1072-1082. DOI: 10.1111/nph.14194

Hogendoorn, K. & Velthuis, H. H. W. (1995). The role of young guards in *Xylocopa pubescens. Insectes Sociaux*, *42,* 427-448.

Hurd, P. D. (1978). An annotated catalog of the carpenter bees (genus *Xylocopa* Latreille) of the Western Hemisphere (Hymenoptera, Anthophoridae). Washington, DC, Smithsonian Institution Press.

Hurd, P. D. & Linsley, E. G. (1964). The squash and gourd bees—genera *Peponapis* Robertson and *Xenoglossa* Smith—inhabiting America north of Mexico (Hymenoptera: Apoidea). *Hilgardia*, *35*, 375-477.

Hurd, P. D., Linsley, E. G. & Michelbacher, A. E., (1974). Ecology of the squash and gourd bee, *Peponapis pruinosa*, on cultivated cucurbits in California (Hymenoptera: Apoidea). Smithson. *Contributions to* Zoology, 1-28.

Hurd, P. D., Linsley, E. G. & Whitaker, T. W. (1971). Squash and gourd bees (*Peponapis*, *Xenoglossa*) and the origin of the cultivated *Cucurbita*. *Evolution*, *25*, 218-234. DOI: 10.2307/2406514

IBPES. (2016). The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination and food production. Potts SG, Imperatriz-Fonseca VL, Ngo HT (eds). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn. 552p

Julier, H. E. & Roulston, T. (2009). Wild bee abundance and pollination service in cultivated pumpkins: farm management: nesting behaviour and landscape effects. *Journal of Economic Entomology*, 102, 563-573.

Keasar, T. (2010). Large carpenter bees as agricultural pollinators. *Psyche: A Journal of Entomology, 2010*, 1-7. DOI:10.1155/2010/927463

Kendall, L. K., Evans, L. J., Gee, M., Smith, T. J., Gagic, V., Lobaton, J. D., Hall, M. A., Jones, J., Kirkland, L., Saunders, M. E., Sonter, C., Cutting, B. T., Parks, S., Hogendoorn, K., Spurr, C., Gracie, A., Simpson, M. & Rader, R. (2021). The effect of protective covers on pollinator health and pollination service delivery. *Agriculture, Ecosystems & Environment*, *319*, 107556. DOI: 10.1016/j.agee.2021.107556

Kishan, T. M., Srinivasan, M. R., Rajashree, V. & Thakur, R. K. (2017). Stingless bee *Tetragonula iridipennis* Smith for pollination of greenhouse cucumber. *Journal of Entomology and Zoology Studies*, *5*, 1729-1733.

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E. & Tscharntke, T. (2014). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B*, *281*, 0132440. DOI: 10.1098/rspb.2013.2440

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences*, *274*, 303-313. DOI: 10.1098/rspb.2006.3721

Klein, A. M., Boreux, V., Fornoff, F., Mupepele, A. C. & Pufal, G. (2018). Relevance of wild and managed bees for human well-being. *Current Opinion on Insect Science*, *26*, 82-88. DOI: 10.1016/j.cois.2018.02.011

Kleinert, A. M. P. & Giannini, T. C. (2012). Generalist Bee Species on Brazilian Bee-Plant Interaction Networks. *Hindawi Publishing Corporation*, *2012*, 1-7. DOI: 10.1155/2012/291519

Khalifa, S. A. M., Elshafiey, E. H., Shetaia, A. A., El-Wahed, A. A. A., Algethami, A. F., Musharraf, S. G., AlAjmi, M. F., Zhao, C., Masry, S. H. D., Abdel-Daim, M. M., Halabi, M. F., Kai, G., Al Naggar, Y., et al. (2021). Overview of Bee Pollination and Its Economic Value for Crop Production. *Insects*, *12*, 688. DOI: 10.3390/insects12080688

Knight, T. M., McCoy, M. W., Chase, J. M., McCoy, K. A. & Holt, R. D. (2005). Trophic cascades across ecosystems. *Nature*, *437*, 880-83.

Koeniger, N. (1976). Interspecific competition between *Apis florea* and *Apis mellifera* in tropics. *Bee World*, *57*, 110-112. DOI: 10.1080/0005772X.1976.11097604

Koppler, K., Vorwohl, G. & Koeniger, N. (2007). Comparison of pollen spectra collected by four different subspecies of the honey bee *Apis mellifera*. *Apidologie*, *38*, 341-353. DOI: 10.1051/apido:2007020

Kumar, P., Baskaran, S., Sundaravadivelan, C., Anburaj, J. & Kuberan, T. (2012). Insect visitors of pumpkin, *Cucurbita maxima* Duch., in relation to temperature and relative humidity. *Journal of Agricultural Technology, 8*, 501-513.

Layek, U., Bisui, S., Mondal, R., Das, N., De, S. K. & Karmakar, P. (2020). Floral traits and chemical cues associated with rock bee (*Apis dorsata* Fabricius) for the host selection in West Bengal, India. *Grana*, *60*, 310-323. DOI: 10.1080/00173134.2020.1823466

Layek, U., Kundu, A., Bisui, S. & Karmakar, P. (2021). Impact of managed stingless bee and western honey bee colonies on native pollinators and yield of watermelon: A comparative study. *Annals of Agricultural Sciences*, *66*, 38-45. DOI: 10.1016/j.aoas.2021.02.004

Lenzi, M., Orth, A. I. & Guerra. T. M. (2005). Ecologia da polinização de *Momordica charantia* L. (Cucurbitaceae), em Florianópolis, SC, Brasil. *Revista Brasileira de Botânica*, *28*, 505-313. DOI: 10.1590/S0100-84042005000300008

Librán-Embid, F., Grass, I., Emer, C., Ganuza, C. & Tscharntke, T. (2021). A plant–pollinator metanetwork along a habitat fragmentation gradient. *Ecology Letters*, *24*, 2700-2712. DOI: 10.1111/ele.13892

Liu, X. & Murata, T. (2010). An efficient algorithm for optimizing bipartite modularity in bipartite networks. *Journal of Advanced Computational Intelligence and Intelligent Informatics*, *14*, 408-415. DOI:10.20965/jaciii.2010.p0408

Lloyd, D. G. & Schoen, D. J. (1992). Self and cross-fertilization in plants. I – Functional dimensions. *International Jounal of Plant Science, 153*, 358-369.

Lobo, J. A. & Méndez, Y. B. (2021). Diversity and foraging patterns of bees on flowers of *Cucurbita pepo* (Cucurbitaceae) in Costa Rica. *Revista de Biología Tropical*, *69*, 494-506. DOI: 10.15517/rbt.v69i2.44076

López-Uribe, M. M., Cane, J. H., Minckley, R. L. & Danforth, B. N. (2016). Crop domestication facilitated rapid geographical expansion of a specialist pollinator, the squash bee *Peponapis pruinosa*. *Proceedings of the Royal Society B: Biological Sciences*, 283(1833), 20160443.

Makkar, G. S. & Chhuneja, P. K. (2016). Bee diversity assemblage on pigeon pea, *Cajanus cajan* along habitat gradient. *Journal of Environmental Biology*, *37*, 1309-1314.

Malerbo-Souza, D. T., Costa, C. F. S., Pimentel, A. C. S., Andrade, M. O., Siqueira, R. A., Silva, R. C. B., Souza, C. M. & Souza, F. G. (2023). Stingless bee *Trigona spinipes* (Hymenoptera: Apidae) behavior on chayote flowers (*Sechium edule*). *Acta Scientiarum. Animal Sciences*, *45*, e56760. DOI: 10.4025/actascianimsci.v45i1.56760

Malepszy, S. & Niemirowicz-Szczytt, K. (1991). Sex determination in cucumber (*Cucumis sativus*) as a model system for molecular biology. *Plant Science, 80*, 39-47. DOI:10.1016/0168-9452(91)90271-9

Mathewson, J. A. (1968). Nest construction and life history of the eastern cucurbit bee, *Peponapis pruinosa* (Apoidea: Hymenoptera). *Journal of the Kansas Entomological Society*, 41 (2), 255-261.

Martín González, A. M., Allesina, S. Rodrigo, A, & Bosch, J. (2012). Drivers of compartmentalization in a Mediterranean pollination network. *Oikos*, *121*, 2001-2013. DOI: 10.1111/j.1600-0706.2012.20279.x

May, M. L. (2019). Odonata: Who they are and what they have done for us lately: classification and ecosystem services of dragonflies. *Insects*, *10*, 1-17. DOI: 10.3390/insects10030062

McGrady, C. M., Troyer, R. & Fleischer, S. J. (2020). Wild Bee visitation rates exceed polination thresholds in commercial *Cucurbita* Agroecosystems. *Journal of Economic Entomology*, *113*, 562-574. DOI: 10.1093/jee/toz295

Mensah, B. A. & Kudom, A. A. (2011). Foraging dynamics and pollination efficiency of *Apis mellifera and Xylocopa olivacea* on *Luffa aegyptiaca* Mill (Cucurbitaceae) in southern Ghana. *Journal of Pollination Ecology*, *4*, 34-38. DOI: 10.26786/1920-7603(2011)6

Moquet, L., Larent, E., Bacchetta, R. & Jacquemart, A-L. (2018). Conservation of hoverflies (Diptera, Syrphidae) requires complementary resources at the landscape and local scales Conservation of hoverflies (Diptera, Syrphidae) requires complementary resources at the landscape and local scales. *Insect Conservation and Diversity,* 11,72-87. DOI: 10.1111/icad.12245

Morimoto, Y., Maundu, P., Fujimaki, H. & Morishima, H. (2005). Diversity of Landraces of the White-flowered Gourd (*Lagenaria siceraria*) and its Wild Relatives in Kenya: Fruit and Seed Morphology. *Genetic Resources and Crop Evolution*, *52*, 737-747. DOI: 10.1007/s10722-004-6119-8.

Morris, W. F., Vázquez, D. P. & Chacoff, N. P. (2010). Benefit and cost curves for typical pollination mutualisms. *Ecology*, *91*, 1276-1285. DOI:10.1890/08-2278.1

Nascimento, V. T., Agostini, K., Souza, C. S. & Maruyama, P. K. (2020). Tropical urban areas support highly diverse plant-pollinator interactions: an assessment from Brazil. *Landscape and Urban Planning*, *198*, 103801. DOI: 10.1016/j.landurbplan.2020.103801

Nepi, M. & Pacini, E. (1993). Pollination, pollen viability and pistil receptivity in *Cucurbita pepo* L. *Annals of Botany*, *72*, 527-536. DOI: 10.1006/anbo.1993.1141

Nicholson, C. C., Ricketts, T. H., Koh, I., Smith, H. G., Lonsdorf, E. V. & Olsson, O. (2019). Flowering resources distract pollinators from crops: model predictions from landscape simulations. *Journal of Applied Ecology*, *56*, 618-628. DOI: 10.1111/1365-2664.13333

Novais, S. M. A., Nunes, C. A., Santos, N. B., D`Amico, A. R., Fernandes, G. W., Quesada, M., Braga, R. F. & Neves, A. C. O. (2016). Effects of a Possible Pollinator Crisis on Food Crop Production in Brazil. *PLoS ONE*, *11*, e0167292. DOI: 10.1371/journal.pone.0167292

Okunlola, B. O., Azeez, S. O. & Faluyi, J. O. (2022). Reproductive biology of two *Lagenaria* (Cucurbitaceae) species. *Botanica*, *28*, 133-144. DOI: 10.35513/Botlit.2022.2.5

Olesen, J. M., Bascompte, J., Dupont, Y. L. & Jordano, P. (2007). The modularity of pollination networks. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 19891-19896. DOI: 10.1073/pnas.0706375104

Patel, S. & Rauf, A. (2017). Edible seeds from Cucurbitaceae family as potential functional foods: Immense promises, few concerns. *Biomedicine & Pharmacotherapy*, *91*, 330-337. DOI: 10.1016/j.biopha.2017.04.090

Patil, A. H. & Jagdale, S. (2021). Nocturnal insect pollinator diversity and species richness in Ridge gourd, *Luffa acutangula*. *Journal of Applied and Natural Science*, *13*, 463-469.

Paulino, C. S., Souza, C. S., Santa-Martinez, E., Almeida, N. M. & Castro, C. C. (2021). Global meta-network of legume crops and floral visitors reveals abundance of exotic bees. *Journal of Applied Entomology*, *146*, 252-261. DOI: 10.1111/jen.12956

Petersen, J. D., Reiners, S., Nault, B. A. (2013). Pollination Services Provided by Bees in Pumpkin Fields Supplemented with Either *Apis mellifera* or *Bombus impatiens* or Not Supplemented. *PLoS ONE, 8,* e69819. DOI:10.1371/journal.pone.0069819

Pinkus-Rendon, M. A., Parra-Tabla, V. & Meléndez-Ramírez, V. (2005). Floral resource use and interactions between *Apis mellifera* and native bees in cucurbit crops in Yucatán, México. *Canadian Entomologist*, *137*, 441-449. DOI:10.4039/n04-043

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution*, *25*, 345-353. DOI: 10.1016/j.tree.2010.01.007

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J. & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to human well-being. *Nature*, *540*, 220-229. DOI: 10.1038/nature20588

Rajan, V. V. & Reddy, P. V. R. (2019). A dead heat in pollination race: A comparative evaluation of the efficiency of a fly (*Chrysomya megacephala*) and a bee (*Apis florea*) in mango pollination. *Journal of Entomology and Zoology Studies*, *7*, 1087-1091.

R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rech, A. R., Agostini, K., Oliveira, P. E., & Machado, I. C. (2014). Biologia da polinização. Rio de Janeiro: Projeto cultural.

Richards, A. J. (1986). Plant Breeding Systems. London: George Allen and Unwin. 529 p.

Rollin, O. & Garibaldi, L. A. (2019). Impacts of honeybee density on crop yield: a meta-analysis. *Journal of Applied Ecology*, *56*, 1152-1163. DOI: 10.1111/1365-2664.13355

Russell, A. L., Buchmann, S. L. & Papaj, D. R. (2017). How a generalist bee achieves high efficiency of pollen collection on diverse floral resources. *Behavioral Ecology*, *28,* 991-1003. DOI: 10.1093/beheco/arx058

Russo, L., Keyzer, C. W, Harmon-Threatt, A. N., LeCroy, K. A. & MacIvor, J. S. (2021). The managed-to-invasive species continuum in social and solitary bees and impacts on native bee conservation. *Current Opinion in Insect Science*, *46*, 43-49. DOI: 10.1016/j.cois.2021.01.001

Ruttner, F., Mossadegh, M. S. & Kauhausen-Kelle, D. (1995). Distribution and variation of size of *Apis florea* F in Iran. *Apidologie*, *26*, 477-486. DOI: 10.1051/apido:19950604

Saade, R. L. (1996). Chayote. *Sechium edule* (Jacq.) Sw. Promoting the conservation and use of underutilized and neglected crops. 8. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy.

Sáez, A., Morales, C. L., Ramos, L. Y. & Aizen, M. A. (2014). Extremely frequent bee visits increase pollen deposition but reduce drupelet set in raspberry. *Journal of Applied Ecology, 51*, 1603-1612. DOI: 10.1111/1365-2664.12325

Sajjad, A. & Saeed, S. (2010). Floral host plant range of Syrphid flies (Syrphidae: Diptera) under natural conditions in southern Punjab, Pakistan. *Pakistan Journal of Botany*, *42*, 1187-1200.

Shah, S. S. A., Hussain, M. I., Aslam, M. K. & Rivera, G. (2014). Natural Products; Pharmacological Importance of Family Cucurbitaceae: A Brief Review. *Mini-Reviews in Medicinal Chemistry*, *14*, 694-705. DOI: 10.2174/1389557514666140820113055

Sihag, R. C. (2021). The red dwarf honey bee (*Apis florea* F.) faces the threat of extirpation in Northwest India. *Ukrainian Journal of Ecology*, *11*, 1-11.

Sinu, P. A., Sibisha, V. C., Nikhila Reshmi, M. V., Reshmi, K. S., Jasna, T. V., Aswathi, K. & Megha, P. P. (2017). Invasive ant (*Anoplolepis gracilipes*) disrupts pollination in pumpkin. *Biological Invasions*, *19*, 2599-2607. DOI: 10.1007/s10530-017-1470-9

Smith, M., Singh, G., Mozaffarian, D. & Myers, S (2015). Effects of decreases of animal pollinators on human nutrition and global health: a modelling analysis. *The Lancet*, 386, 1964-1972. DOI: 10.1016/S0140-6736(15)61085-6

Subhakar, G., Sreedevi, K., Manjula, K. & Eswara Reddy, N. P. (2011). Pollinator diversity and abundance in bitter gourd, *Momordica charantia* Linn. *Pest Management in Horticultural Ecosystems*, *17*, 23-27. DOI: 10.3896/IBRA.1.51.3.02

Tasei, J.-N. & Aupinel, P. (2008). Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris*, Hymenoptera: Apidae). *Apidologie*, *39*, 397-409. DOI: 10.1051/apido:2008017

Tepedino, V. J. (1981). The pollination efficiency of the squash bee (*Peponapis pruinosa*) and the honey bee (*Apis mellifera*) on summer squash (*Cucurbita pepo*). *Journal of the Kansas Entomological Society*, 54 (2): 359-377.

Teppner, H. (2004). Notes on *Lagenaria* and *Cucurbita* (Cucurbitaceae) – Review and New Contributions. *Phyton (Horn, Austria)*, *44*, 245-308.

Theis, N., Barber, N. A., Gillespie, S. D., Hazzard, R. V. & Adler, L. S. (2014). Attracting mutualists and antagonists: Plant trait variation explains the distribution of specialist floral herbivores and pollinators on crops and wild gourds. *American Journal of Botany*, *101*, 1314-1322. DOI: 10.3732/ajb.1400171

Tiitsaar, A., Kaasik, A. & Teder, T. (2013). The effects of seasonally variable dragonfly predation on butterfly assemblages. *Ecology*, *94*, 200-207. DOI: 10.1890/12-0541.1

Tylianakis, J. M. & Morris, R. J. (2017). Ecological Networks Across Environmental Gradients. *Annual Review of Ecology, Evolution, and Systematics*, *48*, 25-48. DOI: 10.1146/annurev-ecolsys-110316-022821

Ullmann, K. S., Meisner, M. H. & Williams, N. M. (2016). Impact of tillage on the crop pollinating, ground-nesting bee, *Peponapis pruinosa* in California. *Agriculture, Ecosystems & Environment*, 232, 240-246

Valido, A., Rodríguez-Rodríguez, M. C. & Jordano, P. (2019). Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific Reports, 9*, 4711. DOI: 10.1038/s41598-019-41271-5

Vázquez, D. P., Morris, W. F. & Jordano, P. (2005). Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters*, *8*, 1088-1094. DOI: 10.1111/j.1461-0248.2005.00810.x

Vidal, M. G., Jong, D., Wien, H. C. & Morse, R. A. (2010). Pollination and fruit set in pumpkin (*Cucurbita pepo*) by honey bees. *Revista Brasileira de Botânica*, *33*, 107-113. DOI: 10.1590/S0100-84042010000100010

Vidhya, A., Suresh, K., Chinniah, C., Krishnamoorthy, V. & Shanthi, M. (2019). Floral diversity and foraging behaviour of little/ dwarf honey bee *Apis florea* F. in horticultural ecosystem, Madurai district, Tamil Nadu. *Indian Journal of Entomology*, *81*, 775-778. DOI: 10.5958/0974-8172.2019.00172.X

Vizentin-Bugoni, J., Maruyama, P. K., Debastiani, V. J., Duarte, L. S., Dalsgaard, B. & Sazima, M. (2016). Influences of sampling effort on detected patterns and structuring processes of a Neotropical plant–hummingbird network. *Journal of Animal Ecology*, *85*, 262-272. DOI: 10.1111/1365-2656.12459

Watts, S., Dormann, C. F., González, A. M. M, & Ollerton, J. (2016). The influence of floral traits on specialization and modularity of plant–pollinator networks in a biodiversity hotspot in the Peruvian Andes. *Annals of Botany*, *118*, 415-429. DOI: 10.1093/aob/mcw114

Wei, T. & Simko, V. (2017). R package "corrplot": Visualization of a Correlation Matrix (Version 0.84). Available in: <https://github.com/taiyun/corrplot>. Accessed in: 10 jan 2021.

Wille, A. (1985). Las abejas *Peponapis* y *Xenoglossa* en Costa Rica y su importancia en la polinización de las *Cucurbita* domésticas. *Revista de Biología Tropical*, *33*, 17-24.

Willis, D. S. & Kevan, P. G. (1995). Foraging dynamics of *Peponapis pruinosa* (Hymenoptera: Anthophoridae) on pumpkin (*Cucurbita pepo*) in southern Ontario. *The Canadian Entomologist*, 127, 167-175. DOI: 10.4039/Ent127167-2

Wolowski, M., Agostini K., Rech A. R., Varassin I. G., Maués M., Freitas L. & Saraiva A. M. (2019). Relatório temático sobre polinização, polinizadores e produção de alimentos no Brasil. Espírito Santo: REBIPP.

Young, H. J. (1988). Neighborhood size in a beetle pollinated tropical aroid: effects of low density and asynchronous flowering. *Oecologia*, *76*, 461-466.

Young, H. J. & Young, T. P. (1992). Alternative Outcomes of Natural and Experimental High Pollen Loads. *Ecology, 73*, 639-647. DOI: 10.2307/1940770

Yurtsever, S., Okyar, Z. & Guler, N. (2010). What colour of flowers do Lepidoptera prefer for foraging? *Biologia*, *65*, 1049-1056. DOI:10.2478/s11756-010-0125-4

Supplement

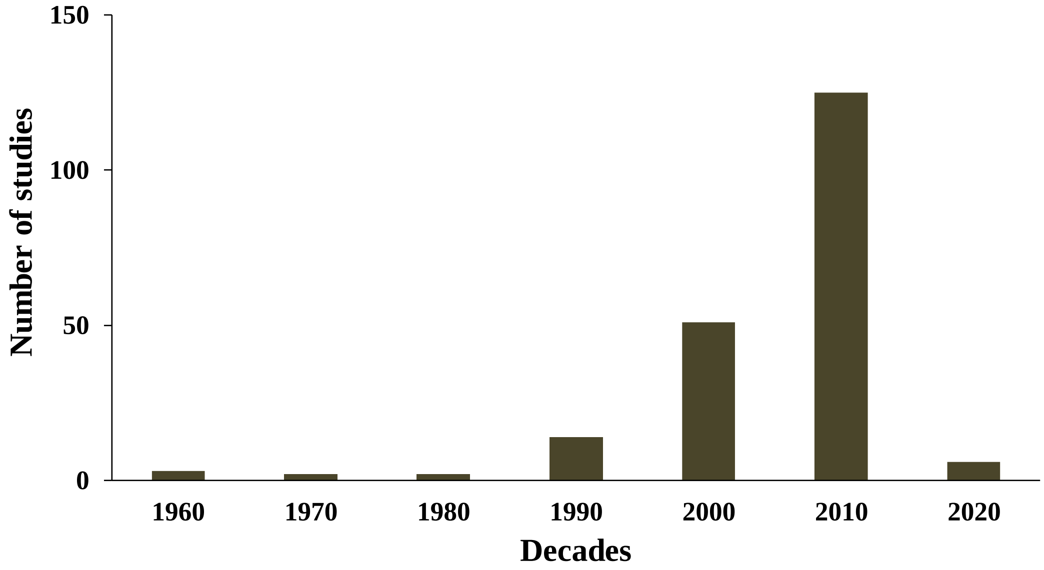

Figure 3. Number of studies that focused on floral visitation of cucurbits of economic importance along decades in the world.

Appendix 1. Pollinators recorded in Cucurbitaceae crops around the world and included in the meta-network.

Number	Species
sp1	*Acraea horta* Linnaeus, 1764
sp2	*Agapostemon angelicus* Cockerell, 1924
sp3	*Agapostemon splendens* (Lepeletier, 1841)
sp4	*Agapostemon texanus* Cresson, 1872
sp5	*Agapostemon virescens* (Fabricius, 1775)
sp6	*Agrius convolvuli* Linnaeus, 1758
sp7	*Amata bicincta* Kollar, 1844
sp8	*Amegilla albocaudata* (Dours, 1869)
sp9	*Amegilla calens* (Lepeletier, 1841)
sp10	*Amegilla cingulata* (Fabricius, 1775)
sp11	*Amegilla zonata* (Linnaeus, 1758)
sp12	*Anadevidia peponis* Fabricius, 1775
sp13	*Andrena ovatula* (Kirby, 1802)
sp14	*Anthidium manicatum* (Linnaeus, 1758)
sp15	*Anthophora bipartita* Smith, 1854
sp16	*Anthophora occidentalis* Cresson, 1869
sp17	*Anthophora urbana* Cresson, 1878
sp18	*Anthophorula chlorina* (Cockerell, 1918)
sp19	*Apis cerana* Fabricius, 1793
sp20	*Apis cerana indica* Fabricius, 1798
sp21	*Apis dorsata* Fabricius, 1793
sp22	*Apis florea* Fabricius, 1787
sp23	*Apis mellifera* Linnaeus, 1758
sp24	*Apis mellifera adansonii* (Latreille, 1804)
sp25	*Apotrigona nebulata* (Smith, 1854)
sp26	*Arthroschista hilaralis* Walker, 1859
sp27	*Astylus atromaculatus* (Blanchard, 1843)
sp28	*Augochlora nigrocyanea* Cockerell, 1897
sp29	*Augochlora pura* (Say, 1837)
sp30	*Augochloropsis metallica* (Fabricius, 1793)
sp31	*Aulacophora femoralis* (Motschulsky, 1857)
sp32	*Aulacophora foveicollis* (Lucas, 1849)
sp33	*Aulacophora palliata* (Schaller, 1783)
sp34	*Blatta orientalis* Linnaeus, 1758
sp35	*Bombus atratus* Franklin, 1913
sp36	*Bombus avanus* (Skorikov, 1938)
sp37	*Bombus bellicosus* Smith, 1879
sp38	*Bombus breviceps* Smith, 1852
sp39	*Bombus californicus* Smith, 1854

sp40	*Bombus diversus* Smith, 1869
sp41	*Bombus ephippiatus* Say, 1837
sp42	*Bombus eximius* Smith, 1852
sp43	*Bombus flavescens* Smith, 1852
sp44	*Bombus friseanus* Skorikov, 1933
sp45	*Bombus griseocollis* (DeGeer, 1773)
sp46	*Bombus haemorrhoidalis* Smith, 1852
sp47	*Bombus hypocrita* Pérez, 1905
sp48	*Bombus hypocrita sapporensis* Cockerell, 1911
sp49	*Bombus ignitus* Smith, 1869
sp50	*Bombus impatiens* Cresson, 1863
sp51	*Bombus impetuosus* Smith, 1871
sp52	*Bombus lapidarius* (Linnaeus, 1758)
sp53	*Bombus lucorum* (Linnaeus, 1761)
sp54	*Bombus motivagus* Smith, 1878
sp55	*Bombus rufofasciatus* Smith, 1852
sp56	*Bombus ternarius* Say, 1837
sp57	*Bombus terrestris* (Linnaeus, 1758)
sp58	*Bombus trifasciatus* Smith, 1852
sp59	*Bombus vosnesenskii* Radoszkowski, 1862
sp60	*Borbo cinnara* Wallace, 1866
sp61	*Calliphora vicina* Robineau-Desvoidy, 1830
sp62	*Camponotus compressus* (Fabricius, 1787)
sp63	*Camponotus sericeus* (Fabricius, 1798)
sp64	*Catopsilia pomona* (Fabricius, 1775)
sp65	*Cephonodes hylas* Linnaeus, 1771
sp66	*Ceratina binghami* Cockerell, 1908
sp67	*Ceratina cognata* Smith, 1879
sp68	*Ceratina hieroglyphica* Smith, 1854
sp69	*Ceratina japonica* Cockerell, 1911
sp70	*Ceratina nanula* Cockerell, 1897
sp71	*Ceratina smaragdula* (Fabricius, 1787)
sp72	*Ceratina viridissima* Dalla Torre, 1896
sp73	*Chrysomya bezziana* Villeneuve, 1914
sp74	*Coccinella septempunctata* Linnaeus, 1758
sp75	*Coccinella transversalis* Fabricius, 1781
sp76	*Coccinella undecimpunctata* Linnaeus, 1758
sp77	*Coelioxys apicata* Smith, 1854
sp78	*Colias eurytheme* Boisduval, 1852
sp79	*Colotis eucharis* (Fabricius, 1775)
sp80	*Cupido comyntas* (Godart, 1824)
sp81	*Dactylurina staudingeri* (Gribodo, 1893)
sp82	*Danaus chrysippus* (Linnaeus, 1758)
sp83	*Delias eucharis* (Drury, 1773)

sp84	*Delta dimidiatipenne* (de Saussure, 1852)
sp85	*Diabrotica speciosa* Germar, 1824
sp86	*Diaphania indica* (Saunders, 1851)
sp87	*Dysphania percota* Swinhoe, 1891
sp88	*Episyrphus balteatus* (De Geer, 1776)
sp89	*Eristalinus aeneus* Scopoli, 1763
sp90	*Eristalinus laetus* (Wiedemann, 1830)
sp91	*Eristalinus obscuritarsis* Meijere, 1908
sp92	*Eristalinus tabanoides* (Jaennicke, 1867)
sp93	*Eucera hamata* (Bradley, 1942)
sp94	*Eupeodes corollae* (Fabricius, 1794)
sp95	*Euphaedra janetta* Butler, 1871
sp96	*Eurema blanda* (Boisduval, 1836)
sp97	*Eurema brigitta* (Stoll, 1780)
sp98	*Eurema hecabe* (Linnaeus, 1758)
sp99	*Eurytides marcellus* (Cramer, 1777)
sp100	*Exomalopsis analis* Spinola, 1853
sp101	*Frieseomelitta nigra* (Cresson, 1878)
sp102	*Gegenes niso* Linnaeus, 1764
sp103	*Gegenes nostrodamus* (Fabricius, 1793)
sp104	*Glyphodes bivitralis* Guenée, 1854
sp105	*Gorgyra johnstoni* Butler, 1893
sp106	*Graphium agamemnon* (Linnaeus, 1758)
sp107	*Halictus confusus* Smith, 1853
sp108	*Halictus farinosus* Smith, 1853
sp109	*Halictus gutturosus* Vachal, 1895
sp110	*Halictus ligatus* Say, 1837
sp111	*Halictus tripartitus* Cockerell, 1895
sp112	*Hemianax ephippiger* (Burmeister, 1839)
sp113	*Henosepilachna vigintioctopunctata* (Fabricius, 1775)
sp114	*Heterotrigona itama* (Cockerell, 1918)
sp115	*Hieroglyphus banian* (Fabricius, 1798)
sp116	*Hippotion celerio* Linnaeus, 1758
sp117	*Homotrigona canifrons* (Smith, 1857)
sp118	*Hylaeus rudbeckiae* (Cockerell & Casad, 1895)
sp119	*Hypotrigona gribodoi* (Magretti, 1884)
sp120	*Illeis cincta* Fabricius, 1798
sp121	*Ischiodon scutellaris* (Fabricius, 1805)
sp122	*Ischnura senegalensis* (Rambur, 1842)
sp123	*Junonia hierta* Fabricius, 1798
sp124	*Junonia iphita* Cramer, 1782
sp125	*Junonia terea* Drury, 1773
sp126	*Lampides boeticus* (Linnaeus, 1767)
sp127	*Lasioglossum cressonii* (Robertson, 1890)

sp128	*Lasioglossum fuscipenne* (Smith, 1853)
sp129	*Lasioglossum halictoides* (Smith, 1858)
sp130	*Lasioglossum hitchensi* Gibbs, 2012
sp131	*Lasioglossum imitatum* (Walker, 1986)
sp132	*Lasioglossum incompletum* (Crawford, 1907)
sp133	*Lasioglossum interruptum* (Panzer, 1798)
sp134	*Lasioglossum leucozonium* (Schrank, 1781)
sp135	*Lasioglossum malachurum* (Kirby, 1802)
sp136	*Lasioglossum pilosum* (Smith, 1853)
sp137	*Lasioglossum tegulare* (Robertson, 1890)
sp138	*Lasioglossum tegulariforme* (Crawford, 1907)
sp139	*Lasioglossum zephyrum* (Smith, 1853)
sp140	*Lasius niger* (Linnaeus, 1758)
sp141	*Lepidotrigona terminata* (Smith, 1878)
sp142	*Leptaulaca fissicollis* Thomson 1858
sp143	*Mantis religiosa* (Linne, 1758)
sp144	*Megachile atrata* Smith, 1853
sp145	*Megachile cephalotes* Smith, 1853
sp146	*Megachile disjuncta* (Fabricius, 1781)
sp147	*Megachile lanata* (Fabricius, 1775)
sp148	*Megachile manyara* Eardley e RP Urban, 2006
sp149	*Melissodes agilis* Cresson, 1878
sp150	*Melissodes bimaculatus* (Lepeletier, 1825)
sp151	*Melissodes lupina* Cresson, 1878
sp152	*Melissodes robustior* Cockerell, 1915
sp153	*Melissodes stearnsi* Cockerell, 1905
sp154	*Melissodes tepaneca* Cresson, 1878
sp155	*Melissodes tepidus* Cresson, 1879
sp156	*Monomorium pharaonis* (Linnaeus, 1758)
sp157	*Musca domestica* Linnaeus, 1758
sp158	*Mylothris agathina* Cramer, 1779
sp159	*Myrmica rubra* (Linnaeus, 1758)
sp160	*Nannotrigona testaceicornis* (Lepeletier, 1836)
sp161	*Neocoenyra gregorii* Butler, 1894
sp162	*Nomia concinna* Smith, 1860
sp163	*Nomia elliotii* Smith, 1875
sp164	*Nomia fulvata* (Fabricius, 1804)
sp165	*Nomia iridescens* Smith, 1853
sp166	*Nomia oxybeloides* Smith, 1875
sp167	*Nomioides minutissimus* (Rossi, 1790)
sp168	*Ocybadistes walkeri* Heron, 1894
sp169	*Oecophylla smaragdina* (Fabricius, 1775)
sp170	*Osmia cornuta* (Latreille, 1805)
sp171	*Osmia lignaria* Say, 1837

sp172	*Pachliopta hector* (Linnaeus, 1758)
sp173	*Papilio demoleus* Linnaeus, 1758
sp174	*Papilio memnon* Linnaeus, 1758
sp175	*Papilio polytes* Linnaeus, 1758
sp176	*Paragomphus lineatus* (Selys, 1850)
sp177	*Paragus crenulatus* Thomson, 1869
sp178	*Paragus serratus* (Fabricius, 1805)
sp179	*Paragus yerburiensis* Stuckenberg, 1954
sp180	*Partamona bilineata* (Say, 1837)
sp181	*Pelopidas mathias* Fabricius, 1798
sp182	*Peponapis fervens* (Smith, 1879)
sp183	*Peponapis limitaris* (Cockerell, 1906)
sp184	*Peponapis pruinosa* (Say, 1837)
sp185	*Phaonia valida* (Harris, 1780)
sp186	*Pieris brassicae* (Linnaeus, 1758)
sp187	*Pieris rapae* (Linnaeus, 1758)
sp188	*Plebeina hildebrandti* (Friese, 1900)
sp189	*Polistes foederata* Kohl, 1898
sp190	*Polistes olivaceus* (Deg., 1773)
sp191	*Polistes stigma* (Fabricius, 1793)
sp192	*Scaptotrigona mexicana* Guérin-Méneville, 1845
sp193	*Syrphus corollae* Fabricius, 1794
sp194	*Tabanus taeniola* Palisot de Beauvois, 1806
sp195	*Tetragonula iridipennis* (Smith, 1854)
sp196	*Tetragonula laeviceps* (Smith, 1857)
sp197	*Trigona fulviventris* Guérin-Méneville, 1845
sp198	*Trigona pallens* (Fabricius, 1798)
sp199	*Trigona spinipes* (Fabricius, 1793)
sp200	*Vespa cincta* De Geer, 1773
sp201	*Vespa orientalis* Linnaeus, 1761
sp202	*Vespa velutina* Lepeletier, 1836
sp203	*Vespula vulgaris* (Linnaeus, 1758)
sp204	*Xenoglossa kansensis* Cockerell, 1905
sp205	*Xenoglossa strenua* (Cresson, 1878)
sp206	*Xylocopa aestuans* (Linnaeus, 1758)
sp207	*Xylocopa basalis* Smith, 1854
sp208	*Xylocopa calens* Lepeletier, 1841
sp209	*Xylocopa dejeanii* Lepeletier, 1841
sp210	*Xylocopa fenestrata* (Fabricius, 1798)
sp211	*Xylocopa flavorufa* (DeGeer, 1778)
sp212	*Xylocopa grisescens* Lepeletier, 1841
sp213	*Xylocopa imitator* Smith, 1854
sp214	*Xylocopa inconstans* Smith, 1874
sp215	*Xylocopa latipes* (Drury, 1773)

sp216	*Xylocopa nigrita* (Fabricius, 1775)
sp217	*Xylocopa nobilis* Smith, 1859
sp218	*Xylocopa olivacea* (Fabricius, 1778)
sp219	*Xylocopa philippinensis* Smith, 1854
sp220	*Xylocopa pubescens* Spinola, 1838
sp221	*Xylocopa tenuiscapa* Westwood, 1840
sp222	*Xylocopa violacea* (Linnaeus 1758)
sp223	*Xylocopa virginica* (Linnaeus, 1771)

CAPÍTULO II

Pollination of zucchini (*Cucurbita pepo*, Cucurbitaceae): helping production improvement through a global review of research approaches, distribution of pollinators and identification of knowledge gaps

Trabalho aceito na revista Ciência Agronômica

Pollination of zucchini (*Cucurbita pepo*, Cucurbitaceae): helping production improvement through a global review of research approaches, distribution of pollinators and identification of knowledge gaps

Isabelle Cristina Santos Magalhães[1]; Marcelo da Rocha Souza[2]; Gerlayne Teixeira de Souza[1]; Isabella Hevely Silva Torquato[1], André Maurício Melo dos Santos[3]; Cibele Cardoso de Castro[1,2*]

[1] Programa de Pós-Graduação em Biodiversidade, Universidade Federal Rural de Pernambuco, Rua Manoel de Medeiros s/n, 52171-900, Recife, Pernambuco, Brazil

[2] Universidade Federal do Agreste de Pernambuco, Avenida Bom Pastor, s/n, 55292272, Garanhuns, Pernambuco, Brazil.

[3] Universidade Federal de Pernambuco, Rua Alto do Reservatório, s/n, 55608-680, Vitória de Santo Antão, Pernambuco, Brazil

*Corresponding author cibele.castro@ufape.edu.br

Running title: Global review on zucchini pollination

Abstract

Crop pollination is indispensable for global food security. Studies that summarize the knowledge about pollination of specific crops are relevant because they identify pollinators' distribution, guide pollinators management and conservation policies, and shed light on knowledge gaps. Zucchini production is essentially dependent on pollinators, is cultivated in several countries and has great economic importance. The objective of this study was to integrate global data on zucchini pollination that are available in the main scientific repositories and answer the following questions: 1) How are studies on zucchini pollination distributed in time and space? 2) What are the topics addressed and what are the trends of the results? 3) Who are the pollinators and how are they globally distributed? 4) What are the gaps of knowledge? We performed a systematic literature review, analyzed the distribution of studies in decades and regions (temperate versus tropical), built a network of countries and pollinators and compared data on the efficiency of specific pollinators. Studies were conducted in 16 countries distributed in almost all continents, mostly in temperate regions, and more than a half in the USA. Most studies investigated the frequency and diversity of floral visitors. Other approaches were investigations on dependence of production on pollination, influence of the landscape on pollination and efficiency of specific pollinators. Zucchini flowers fed 116 species of pollinators, especially bees. Six countries had almost exclusive groups of native pollinators. *Apis Bombus* and *Peponapis* were the most frequently recorded bees. Areas with high habitat diversity improve pollination. There was a significant difference in productivity when pollination was carried out by bees when compared to production by Syrphidae. Many countries had almost exclusive groups of native pollinators, among which it is possible to find efficient and manageable species, capable of replacing (even partially) exotic pollinators. The main gaps of knowledge found were 1) The determination of which native, manageable pollinators are efficient for maximum zucchini production 2) The investigation of how pollination influence fruit nutritional composition and seed quality, and 3) The identification of pollinators until the species level.

Keywords: Apidae, bees, crop pollination, ecosystem services, global food security, pollinators decline, vegetables.

Introduction

Vegetables and fruits are largely pollinator-dependent crops that represent the most important sources of micronutrients in human diet (Garibaldi et al., 2022; Porto et al., 2021; Smith et al., 2015). Due to the global pollinators decline (Potts et al., 2010), an evident decrease in world agricultural production has occurred along the last decades (Ellis et al., 2015; Smith et al., 2015), threatening global nutritional security (Chaplin Kramer et al., 2014; IPBES, 2016; Peixoto et al., 2022). Therefore, many studies on crop pollination seek to understand the influence of pollinators on quantitative and qualitative aspects of production, their economic valuation, as well as to estimate the contribution of agricultural crops to the resilience of pollination services (IPBES, 2018; Klein et al., 2018). Few studies, however, synthesize global data on the pollination of specific crops. They are relevant because identify the distribution of pollinators, guide pollinators management and conservation policies, and shed light on knowledge gaps.

The zucchini (*Cucurbita pepo* L., Cucurbitaceae) is an example of vegetable crop with great economic importance, whose production is essentially dependent on pollinators (Giannini, Cordeiro, et al., 2015), and whose data on pollination were not compiled so far. Like other Cucurbitaceae species, zucchini individuals have staminate (male) and pistillate (female) flowers (i.e., monoecy., García et al., 2020; Hoehn et al., 2008). Therefore, fruit and seed production rely on animal pollinators that transport the large and heavy pollen grains to the sticky pistil of the female flowers (Rech et al., 2014). The world production of zucchini together with other cucurbits is around 35 million tons, and plantations occupy approximately two million hectares (FAO, 2022). Although cross-pollination is mandatory for zucchini, the economic valuation of pollinators is still unknown (Wolowski et al., 2019).

Because flowers are large and produce abundant and easily accessible nectar and pollen (Nicodemo et al., 2009), they are visited by a great diversity of insects; however, bees are the main pollinators (Giannini, Boff, et al., 2015). The bees of Apini tribe are the most expressive, due to their higher floral visitation rate (Giannini, Boff, et al., 2015). The presence of Apini bees improve several commercially important characteristics of fruits and seeds, mainly size and weight, ensuring greater economic market value (Gemmill-Herren, 2016; Klein et al., 2007).

Considering the high dependence of zucchini production on pollinators, its economic relevance and the absence of studies that synthetize the data on zucchini pollination, this study aims to integrate global data on zucchini pollination to answer the following questions: 1) How are studies on zucchini pollination distributed in time and space? 2) What are the topics addressed in the studies and what are the trends of the results? 3) Who are the pollinators and how are they distributed? 4) What are the gaps of knowledge?

Materials and methods

Systematic review of the literature

To obtain data on zucchini pollination, we conducted a systematic literature review (from 1970 to 2021) using the repositories Web of ScienceTM (www.webofknowledge.com), Google Scholar® (www.scholar.google.com), Scielo (www.scielo. org) and Scopus (www.scopus.com) with the following search terms: ((“zucchini” OR “Cucurbita pepo”) AND (“pollination” OR “pollinator” OR “floral visitor” OR “floral biology” OR “breeding system"). The inclusion criteria of studies were to clearly bring the information that the species behaved as a pollinator and have the identification at the species level and to be written in English. This initial search returned 16.000 studies, but only 51 met the inclusion criteria (Fig. 1).

For each study, we extracted the year of publication, country of data collection, main questions, and type of study area (open field or greenhouse), pollinators’ species and main results. The update of the scientific names of the floral visitors was verified, through the Moure's Bee Catalog (moure.cria.org.br/) and the Global Names Resolver (resolver.globalnames.org/).

We used One-way (comparing the levels of one only variable) post-hoc pairwise chi-square analysis to check for significant differences in the frequencies of decade (≥ 2010, < 2010), regions (tropical, temperate), environments (open, closed, both) and study categories (pollinators’ diversity and frequency of visits, reproductive experiments, influence of landscape on pollination and tests of pollinator efficiency). The post-hoc pairwise was performed using the function *pairwise Nominal Independence* of the *r companion* package (Mangiafico, 2022).

To analyze the geographical distribution of pollinators, a network was created from a weighted matrix, with countries in rows and pollinators as columns. The cells were filled in with the number of studies that recorded the occurrence of a pollinator species in each country. The thickness of the edges in the net indicates the weight (number of studies). To assess the role of nodes in the network structure, we calculated the following centrality metrics: degree, which indicates how much a node is connected to other nodes in the network (Rodrigues, 2019); and betweenness, which describes the importance of a node as a connector between different parts of the network (Freeman, 1979). The network was designed using the Fruchterman-Reingold algorithm (Fruchterman & Reingold, 1991) in the Igraph package of the R software (R Core Team, 2023).

Results

A total of 51 studies met the inclusion criteria, dating from 1981 to 2021. The number of studies published annually increased significantly after the 2000s (38 studies or 74.50%), with the 1980s to 2000 the least expressive (13 studies or 25.49%; $\chi 2 = 12.255$; df = 1; $p < 0.01$; Fig. 2). Data were collected in 16 countries (Austria, Bangladesh, Brazil, Canada, China, Costa Rica, Côte d'Ivoire, Ghana, Guatemala, Italy, Nepal, Pakistan, Saudi Arabia, Spain, United Kingdom, and United States, with the largest number in the United States (26 studies or 50.98%; Fig. 2). The proportion of studies was significantly higher in temperate regions (37 studies or 72.54%), conducted especially in open plantations, followed by closed environment and both situations. Data of studies of tropical regions (14 studies or 27.45%; $\chi 2 = 10.373$; df = 1; $p < 0.01$; Fig. 2) were all conducted in open plantations ($\chi 2 = 59.765$; df = 2; $p < 0.01$).

Regarding the main approaches of the studies, most of them evaluated the frequency and diversity of pollinators (41 studies or 80.39%), evaluation of reproductive requirements by comparing the production between natural and hand pollinations (11 studies or 21.56%), influence of the landscape on pollination in open plantations (8 studies or 15.68%) and evaluation of the influence of a specific pollinator on production (4 studies or 7.84%; $\chi 2 = 53.625$, df = 3, $p < 0.01$). The main results are presented below.

Most studies that investigated the frequency and diversity of floral visitors associated visitation rate with various aspects of foraging behavior, such as visit time, pollen collection vs. nectar or nectar theft (31 studies or 75.60%), abundance and density of pollen grains on the stigma after different numbers of visits (one, two, four, eight and twelve; 19 studies or 46.34%). A total of 38 studies (74,50%) recorded *Apis* species and, among them, 34 recorded *A. mellifera*, three recorded *A. cerana* and two *A. dorsata*. A smaller number of studies addressed other bee species, including *Bombus* spp. (24 studies or 47.05%), with *B. impatiens* being the most recorded (11 studies), followed by *B. terrestris* (four studies). Species of *Peponapis* (22 studies or 43.13%) included *P. pruinosa* (17 studies), *P. apiculata*, *P. fervens* and *P. utahensis* (two studies), and *P. limitaris* (one study).

Studies that evaluated the reproductive requirements (11 studies or 21.56%). Among them, nine were focused on natural pollination and observed that bee pollination improved production both in quantity (number) and in quality (weight, length, and diameter) of fruits and seeds. Two studies that compared fruit set between manual cross- and natural pollination did not find differences.

The studies that evaluated the influence of landscape on zucchini pollination and production tested for different distances from areas with high diversity of habitats (i.e., natural and seminatural vegetation cover). Most of them (seven or 63,63%) observed that plantations distant from 300 to 2000m to those areas had the frequency of visits by *B. impatiens*, *A. mellifera* and *Peponapis* spp improved, but did not test for the impact on production. A study investigated the effects of the use of chemicals (insecticides and fungicides) on pollen and bees in areas 2km far from the plantings, rom the plantations, and observed high concentrations of chemicals in pollen grains, as well as that insecticides were approximately 100 times more dangerous for bees than fungicides, exponentially decreasing the visitation of native bees.

The four studies that investigated the efficiency of specific pollinators on production included Apidae (bees), Halictidae (bees) and/or Syrphidae (flies). Three studies investigated *Apis* species in open environment. One of them compared *A. dorsata*, (which is considered the best pollinator of zucchini in Pakistan), with 23.33% of fruit set, with *Eristalinus laetus* (6.,66%), *E. aeneus* (6.66%), *Lasioglossum* sp1 (10%), *Lasioglossum* sp2 (13.33%), *Halictus* sp. (20%) and *Nomia* sp. (36.66%). The last one was considered the best pollinator, followed by *Halictus* sp., while the other pollinator species were not statistically significant.The second study compared *A. mellifera* with *Bombus impatiens* and *Peponapis pruinosa* and observed that the second bee deposited three times more pollen grains onto stigmas in a single visit when compared to the other two species, reaching 64.7%, while *A mellifera* reached around 18% and *P. pruinosa* 10%. The third study compared *A. mellifera* with *P. pruinosa* and found that although the fruit set after pollination by *A. mellifera* was higher than other species, most flowers require more than one visit to reach 30 to 50% of fruit set. Although *P. pruinosa* is the cucurbit bee, rarely a single visit is enough to produce fruit, especially when there is another competing pollinator in the growing area.

Considering all studies that tested the efficiency of a specific pollinator, there was a significant difference in productivity when pollination was carried out by bees, when compared to production by Syrphidae ($\chi 2 = 35.095$, df = 9, $p < 0.01$). Among the most efficient species, *P. pruinosa* (52.63%) and *B. impatiens* (41.46%) stand out. *Bombus impatiens* was more efficient than *Eristalinus aeneus* ($p = 0.0221$) and *E. megacephalus* ($p = 0.0221$) for fruit set. Similarly, the efficiency of *P. pruinosa* was significantly higher than *E. aeneus* ($p = 0.0104$), *E. megacephalus* ($p = 0.0104$) and *Lasioglossum* sp.1 ($p = 0.0432$). No study compared the influence of pollination on chemical aspects of fruits and in seed germination. A total of 116 species of pollinators was recorded (Appendix 1), distributed in four orders (Coleoptera, Diptera, Hymenoptera and Lepidoptera, Fig. 3), eight families and 37 genera (Fig. 3). Most species are included in Hymenoptera genera (106 or 91.37%), exclusively bees, and distributed in North, Central and South America, Europe, Africa, and Asia (Fig. 3). Diptera species has been recorded in Asia and Europe, Coleoptera only in North America, and Lepidoptera only in Asia. The most representative families were Apidae (bees, 64 species or 55.17%), followed by Halictidae (bees, 39 species or 33.62%). The genus *Apis* had the widest geographical distribution of records from studies in North America, Europe, and Asia. *Bombus* records were also broadly distributed, mainly in North America and Europe, and less frequent in South America. *Peponapis* was recorded mainly in the North America. Other less frequent genera were widely distributed in North and Central America (Fig. 3).

Among the countries that presented the largest number of connections in the network (Fig.4) are the United States (betweenness = 0.825), with 8% of its species recorded in other 14 countries, and the United Kingdom (betweenness = 0.174), with 40% of the species also observed in 12 countries. *Apis mellifera* presented the widest geographic distribution of records, being registered in 13 countries and, consequently, obtained the highest connection value (betweenness = 0.822), followed by the bees *Augochloropsis metallica* (0.122, two countries) and *B. impatiens* (0.012, three countries).

The network revealed that the United States had the highest number of pollinator species (50 species or 43.10%, degree = 50), most of them (46) being exclusive. The four species shared with other countries were *A. mellifera*, *Peponapis pruinosa*, *Bombus impatiens* and *Augochloropsis metallica*; the first three were the most recorded in 19, 15 and nine studies, respectively. In Guatemala, 22 species (degree = 22) were observed in only one study, and, in Costa Rica, 20 species (degree = 20), seven of which were recorded in two studies, and six shared with Guatemala. China (11 species; degree = 11) and Brazil (10 species, degree = 10) presented nine exclusive species each, sharing only *A. mellifera* with other countries. Pakistan has recorded nine unique species (degree = 9). Five species were identified in the United Kingdom (degree = 5), with three exclusive species, and *A. mellifera* (three studies) and *B. terrestris* (two studies) being the most recorded. Côte d'Ivoire (one exclusive species) and Spain recorded three species (degree = 3), with similar records for *A. mellifera* and *B. impatiens* (one study each). In Austria, two species (*A. mellifera* and *B. terrestris*; degree = 2) were observed in only one study. The other countries recorded only one species (degree = 1): *A. mellifera* in Bangladesh, Ghana, Italy and Saudi Arabia, *P. pruinosa* in Canada, and *A. dorsata* in Nepal.

Discussion

Our review showed that 90% of studies on zucchini pollination were carried out from the 2000s onwards, probably due to the growing concern about the global pollinator crisis that resulted in a significant reduction in the diversity, density, and distribution of pollinators around the world, compromising human food security (Aizen et al., 2022; Bartomeu et al., 2018; Novais et al., 2016). Crops more dependent on pollinators tend to be more affected by the pollinator crisis (Garibaldi et al., 2011; Klein et al., 2007) due to insufficient quantity and quality of pollen delivered to the stigmas of cultivated plants, or pollen limitation (Freitas et al., 2016; Vaissière et al., 2011). For this reason, studies are being carried out with the aim of mitigating this crisis (IBPES, 2016; Shivanna et al., 2020). There are strong evidence of pollinator declines in the United States since 1947, with a loss of 59% of bee colonies (Stokstad, 2007), and in Europe since 1985 with a loss of about 25% (Potts et al., 2010). This fact may explain the predominance of studies originating in North America, especially in the United States, and in Europe.

The higher number of studies observed in the US is not explained by its production, since it has the fifth gross production value in the world (FAOSTAT, 2022). It is important to note that zucchini is native to North America, where there is strong evidence that it was domesticated at least twice, being in Mexico more than 10,000 years ago and in the United States more than 4,000 years ago, later domesticated in various locations on the North American continent (Paris, 2016). The predominance of studies in the US clearly influenced the higher proportion of studies in temperate regions when compared to tropical ones. It is important to note that the climate of temperate regions allows the maximum production of the harvest, with higher quality fruits (Salehi et al., 2019).

The predominance of studies conducted in open plantations may be explained by the fact that this system allows for until 70% increase in zucchini yield due to free access of a higher diversity and frequency of pollinators to flowers (Waters & Taylor, 2006) when compared to indoor cultivation, which tend to have insufficient pollination, causing loss in productivity (Cruz & Campos, 2009; Formisano et al., 2020). Pollination of crops in open fields is strongly favored by the landscape, which provides a variety of floral resources (pollen, nectar, and oil sources) and nesting sites for pollinators (Fijen et al., 2019; Garibaldi et al., 2013; Garibaldi et al., 2016; Parra-Tabla et al., 2017). It is important to note that agricultural cultivation carried out indoors is gaining prominence worldwide, allowing the production of high-quality fruits throughout the year, in addition to reducing pest attacks and, consequently, reducing the use of pesticides (Campeche et al., 2017; Shamshiri et al., 2018).

The fact that the global distribution of zucchini pollination studies is heavily concentrated on *Apis* and *Bombus* species in North America is explained by the fact that those bees constitute the main group of managed pollinators in this continent (Ghazoul, 2015; Goulson, 2003; Klein et al., 2007; Millard et al., 2020). *Apis* and *Bombus* are known to guarantee the production of zucchini fruits and seeds with greater quantity and quality (Krug et al., 2010; Nicodemo et al., 2009; Roubik, 2018; Vidal et al., 2010). It is interesting to note that the growing number of studies with species of the genus *Apis* occurred after infestations by the parasite *Varroa destructor* in the United States in the 1980s (IBPES, 2016; Oldroyd, 1999). The rapid increase of studies with the genus *Bombus* occurred in the late 1980s with the first commercialization of species for pollination of crops (Velthuis & van Doorn, 2006). From that period on, other genera of pollinators were frequently studied (Millard et al., 2020).

The most frequent bee species in the studies (*A. mellifera, B. impatiens, P. pruinosa*) were recorded in tropical and temperate regions (Koné et al., 2019; Malerbo-Souza et al., 2019; Phillips & Gardine, 2015). The high frequency of *A. mellifera* is closely related to the fact that it is a generalist species, widely managed for bee products and crop pollination, having considerable economic value (Delaplane & Mayer, 2000; Kevan, 1997; Wolowski et al., 2019). It is considered as an efficient pollinator for zucchini flowers, from which it collects nectar and pollen and increases fruit production, reaching almost 100% after 12 visits (Vidal et al., 2010; Artz et al., 2011; Petersen et al., 2013). However, the presence of this species causes several negative impacts on the ecosystem, interfering with the relationships between plants and native pollinators, causing a reduction in their diversity, making the vast plant-pollinator interactions impossible and, consequently, causing the failure of the reproductive system of the plants that depend on these animals (Valido et al., 2019).

Although world agricultural production depends on pollination by *Apis*, it is highly recommended that countries seek pollinators that can replace it even partially, giving preference to native, manageable, and efficient species (IBPES, 2016). The network revealed that several countries have exclusive native pollinators that could met those criteria, such as *Agapostemon*, *Euglossa*, *Eulaema*, *Exomalopsis*, *Caenaugochlora*, *Halictus*, *Megalopta*, *Melipona*, *Nannotrigona*, *Tetragona*, *Thygater*. Some genera have been already managed in agriculture, such as sch as *Melipona* (Mascena et al., 2018), *Nannotrigona* (Silva & Gimenes, 2014) and *Tetragona* (Oliveira-Junior et al., 2022). The large number of pollinator species recorded in several countries reinforces the importance of maintaining the diversity of these animals for the maintenance of agricultural production. Thus, in addition to seeking native and manageable pollinators through pollinator efficiency studies, it is essential to conserve environments with a high diversity of habitats, which are known to maintain pollinator populations around the world.

Despite having a lower frequency, *B. impatiens* is considered a highly efficient pollinator in zucchini (Artz et al., 2011; Artz & Nault, 2011; Petersen et al., 2014). The efficiency of this species is directly related to its body size (ranging from 1 to 4 cm in length) and the dense coat that allows large amounts of pollen to be carried during a visit (Goulson, 2010; Herrmann et al., 2018), ensuring similar fruit set open pollination when flowers are visited four to eight times (Artz & Nault, 2011).

The bee *P. pruinosa* is a specialist pollinator of Cucurbitaceae crops, mainly squash (Artz et al., 2011; Skidmore et al., 2019), and it is considered one of the most abundant native bees in *Cucurbita* crops in the United States (Sampson et al., 2007; Shuler et al., 2005), being found in 93% of the studies carried out in the region. Although is considered an efficient pollinator, capable of sustaining most of the zucchini production, when the flowers are visited seven times (Cane et al., 2011), pollination by this species may be less efficient when compared to other species, since the frequency of visits to pistillate flowers is much lower, consequently reducing fruit weight and seed formation (Artz & Nault, 2011; Petersen et al., 2014).

The greater proportion of studies investigating the frequency and diversity of floral visitors is justified by its importance as a basis for understanding how much the crop depends on pollinators, how many visits are necessary for a desirable production, as well as to guide pollinator management. The assessment of pollination deficits in production through natural and cross-pollination experiments, although little addressed in studies with zucchini, has been gaining increasing attention in the literature as it allows providing an estimate of pollination needs for pollinator-dependent crops (Petersen et al., 2014). In addition, such studies contribute to the identification of other factors that can influence production in addition to the lack of pollen (Vidal et al., 2010), promoting an increase in agricultural resilience and bringing economic returns (Knapp & Osborne, 2017).

Studies testing the efficiency of different species of pollinators in the production are still very limited, even being accepted that this knowledge helps in identifying alternative pollinators (Artz & Nault, 2011) and in determining the ideal number of visits for maximum crop yield (Sihag, 2018). Despite the importance of native bees for zucchini productivity (Enríquez et al., 2015), only a few studies have documented the performance of these pollinators. Thus, new studies are needed to conserve and develop management strategies for native pollinators (Ali et al., 2014; Malerbo-Souza et al., 2019). Studies that evaluate the landscape around zucchini plantations, despite being scarce, have become a trend in research on agricultural crops, since it directly influences production, as commented above. In addition, it has been proven that the amount and proximity of native vegetation, for example, are determining factors for the increase in bee populations and the effectiveness of pollination services in zucchini crops (Petersen & Nault, 2014).

Although zucchini has antioxidants that benefit human health in its fruits (Boschi, 2015), it is worth noting that none of the works carried out studies on the influence of pollinators on the chemical characteristics of zucchini fruits. It is known that pollinators may alter chemical composition of fruits (Cruz, 2009; Klatt et al., 2014; Vergara & Fonseca-Buendía, 2012; Baronio et al., 2021). Also, no studies investigated the influence of pollinators in seed germination. This is especially important for crops that are cultivated through seeds, as the zucchini. Pollinators maximize seed production in more than 40 crops worldwide (Garibaldi et al., 2013) and in at least ten Brazilian crops (Giannini, Cordeiro, et al., 2015) and may also positively influence seed germination (Kevan & Eisikowitch, 1990).

Conclusions

We reviewed studies related to the influence of pollinators on zucchini crops, how they are distributed, who are the pollinators and what are the main gaps in knowledge, data that will help in the development and improvement of strategies for the management and pollinator conservation. Most studies were conducted from the 2000s onwards, mainly in temperate regions and in open environments. The study approaches refer to the evaluation of the frequency and diversity of pollinators in the production, comparison of the production between natural pollination and manual pollination, influence of the landscape on the pollination in open plantations and evaluation of the influence of a specific pollinator in the production. Bees behaved as the main pollinators, followed by other insects such as flies, beetles and butterflies. The predominance of studies with *Apis*, *Bombus* and *Peponapis* is probably related to their economic importance. Studies regarding reproductive requirements in different regions by using controlled crosses are needed to help in the maximum yield. In addition, studies evaluating the efficiency of different pollinator species in the production would help in the elaboration of management and conservation practices. There is still a need to assess the influence of pollinators on the chemical aspects of the fruits and in seed germination.

Zucchini flowers fed 116 species of pollinators, especially bees, followed by flies, beetles, and butterflies. Six countries had almost exclusive groups of pollinators. *Apis* species was recorded in all countries and, together with *Bombus* and *Peponapis*, formed the most frequently recorded bees. Studies that investigated the influence of the landscape on pollination found that area with high habitat diversity improve pollination. Considering all studies that tested the efficiency of a specific pollinator, there was a significant difference in productivity when pollination was carried out by bees when compared to production by Syrphidae. The main gaps of knowledge found were 1) The determination of which native, manageable pollinators are efficient for maximum zucchini production 2) The investigation of how pollination influence fruit nutritional composition and seed quality, and 3) The identification of pollinators until the species level.

Competing Interests

Authors have no competing interests.

Funding

Isabelle Cristina and Gerlayne Teixeira received financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Financing Code 001), Marcelo Rocha from Fundação de Amparo à Ciência e Tecnologia de Pernambuco (BIC-0700-2.03/21), and Cibele Cardoso from Conselho Nacional de Desenvolvimento Científico e Tecnológico (research grant: 428303/2018-8, fellowship: 312372/2021-3).

Aknowledgements

We thank the Programa de Pós-Graduação em Biodiversidade of the Universidade Federal Rural de Pernambuco and the Universidade federal do Agreste de Pernambuco for the institutional support. The authors do not have conflicts of interest.

Author Contribution

Isabelle Cristina Santos Magalhães, Marcelo Rocha Souza and Gerlayne Teixeira de Souza carried out the research.

Isabella Hevely Silva Torquato and André Maurício Melo dos Santos analyzed the data and performed statistical analyses.

Cibele Cardoso de Castro coordinate the study and obtained funding.

All authors wrote the manuscript.

All authors read and approved the manuscript.

Ethical Approval

N/A

Data Availability Statement

All data used in this study will be available at Figshare.

References

Aizen, M. A., Garibaldi, L. A., & Harder, L. D. (2022). Myth and reality of a global crisis for agricultural pollination. *Ecología Austral*, 32, 698-715. https://doi.org/10.25260/EA.22.32.2.1.1875

Ali, M., Saeed, S., Sajjad, A., & Bashir, M. A. (2014). Exploring the best native pollinators for pumpkin (*Cucurbita pepo*) production in Punjab, Pakistan. *Pakistan Journal of Zoology*, 46(2), 531-539.

Artz, D. R., & Nault, B. A. (2011). Performance of *Apis mellifera*, *Bombus impatiens*, and *Peponapis pruinosa* (Hymenoptera: Apidae) as pollinators of pumpkin. *Journal of Economic Entomology*, 104(4), 1153-1161. https://doi.org/10.1603/EC10431

Artz, D. R., Hsu, C. L., & Nault, B. A. (2011). Influence of honeybee, *Apis mellifera*, hives and field size on foraging activity of native bee species in pumpkin fields. *Environmental Entomology*, 40(5), 114-1158. https://doi.org/10.1603/EN10218

Baronio, G. J., Souza, C. S., Silva, N. N. A., Moura, N. P., Leite, A.V., Santos, A. M. M., Maciel, M. I. S., & Castro, C. C. (2021). Different visitation frequencies of native and non-native bees to vines: how much vegetation is necessary to improve fruit production? *Plant Biology*, 23, 923-930. https://doi.org/10.1111/plb.13327

Bartomeus, I., Stavert, J. R., Ward, D., & Aguado, O. (2018). Historical collections as a tool for assessing the global pollination crisis. *Philosophical Transactions of the Royal Society B*, 374(1763), 20170389. https://doi.org/10.1098/rstb.2017.0389

Boschi, K. (2015). Caracterização das propriedades químicas e antioxidantes da semente, germinados, flores, polpa e folha desenvolvida de abóbora (*Cucurbita pepo* L.). Dissertação de MS, Escola Superior Agrária de Bragança, Bragança.

Campeche, L. F. S., Franca, R. J. F., Leitão, M. M. V. B. R., Silva, J. S., & Paz, V. P. S. (2017). Microclima e evapotranspiração de tomate em dois sistemas de produção no vale do São Francisco. *Agrometeoros*, 25(1), 133-142.

Cane, J. H., Sampson, B. J., & Miller, S. A. (2011). Pollination value of male bees: the specialist bee *Peponapis pruinosa* (Apidae) at summer squash (*Cucurbita pepo*). *Environmental Entomology*, 40(3), 614-620. https://doi.org/10.1603/EN10084

Chaplin-Kramer, R., Dombeck, E., Gerber, J., Knuth, K. A., Mueller, N. D., Mueller, M., Ziv, G., & Klein, A. M. (2014). Global malnutrition overlaps with pollinator-dependent micronutrient production. *Proceedings of the Royal Society B* 281(1794), 20141799. https://doi.org/10.1098/rspb.2014.1799

Cruz, D. O., & Campos, L. A. O. (2009). Polinização por abelhas em cultivo protegido. *Revista Brasileira de Agrociência*, 15(1-4), 5-10.

Delaplane, K. S., & Mayer, D. F. (2000). Crop pollination by bees. CABI Publishing, Nova York, Estado Unidos.

Ellis, A. M., Myers, S. S., & Ricketts, T. H. (2015). Do Pollinators contribute to nutritional health? *PLoS One*, 10(1), e114805. https://doi.org/10.1371/journal.pone.0114805

Enríquez, E., Ayala, R., Gonzalez, V. H., & Núñez-Farfán, J. (2015). Alpha and beta diversity of bees and their pollination role on *Cucurbita pepo* L. (Cucurbitaceae) in the Guatemalan cloud forest. *The Pan-Pacific Entomologist*, 91(3), 211-222. https://doi.org/10.3956/2015-91.3.211

FAOSTAT. 2022. Food and Agriculture Organisation of the United Nation. (http://faostat.fao.org/)

Fijen, T. P. M., Scheper, J. A., Boom, T. M., Janssen, N., Raemakers, I., & Kleijn, D. (2018). Insect pollination is at least as important for marketable crop yield as plant quality in a seed crop. *Ecology Letters*, 21(11), 1704-1713. https://doi.org/10.1111/ele.13150

Fijen, T. P., Scheper, J. A., Boekelo, B., Raemakers, I., & Kleijn, D. (2019). Effects of landscape complexity on pollinators are moderated by pollinators' association with mass-flowering crops. *Proceedings of the Royal Society B*, 286(1900), 20190387. https://doi.org/10.1098/rspb.2019.0387

Formisano, L., El-Nakhel, C., Corrado, G., Pascale, S., & Rouphael, Y. (2020). Biochemical, physiological, and productive response of greenhouse vegetables to suboptimal growth environment induced by insect nets. *Biology*, 9(12), 432. https://doi.org/10.3390/biology9120432

Freeman, L. C. (1977). A set of measures of centrality based on Betweenness. *Sociometry* 40, 35-41. https://doi.org/10.2307/3033543

Freitas, B. M., Vaissière, B. E., Saraiva, A., & Carvalheiro, L. G. **(**2016). Identifying and assessing pollination deficits in crops. In: Pollination services to agriculture - Sustaining and enhancing a key ecosystem service, eds. B. Gemmill-Herren, 17-32. New York: Routeledge.

Fruchterman, T. M. J., Reingold, E. M., & Wiley, J. (1991). Graph Drawing by Force-Directed Placement. Softw. Pract. Exp., 21, 1129-1164.

García, A., Aguado, E., Garrido, D., Martínez, C., & Jamilena, M. (2020). Two androecious mutations reveal the crucial role of ethylene receptors in the initiation of female flower development in *Cucurbita pepo*. *The Plant Journal*, 103(4), 1548-1560. https://doi.org/10.1111/tpj.14846

Garibaldi, L. A., Aizen, M. A., Klein, A. M., Cunningham, S. A., & Harder, L. D. (2011). Global growth and stability of agricultural yield decrease with pollinator dependence. *PNAS*, 108(14), 5909-914. https://doi.org/10.1073/pnas.1012431108

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., Kremen, C., Carvalheiro, L. G., Harder, L. D., Afik, O., et al. (2013). Wild pollinators enhance fruit set of crops regardless of honeybee abundance. *Science*, 339(6127), 1608-1611.

Garibaldi, L. A., Carvalheiro, L. G., Vaissiere, B. E., Gemmill-Herren, B., Hipolito, J., Freitas, B. M., & Zhang, H. (2016). Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science*, 351(6271), 388-391.

Garibaldi, L. A., Gomez Carella, D. S., Nabaes Jodar, D. N., Smith, M. R., Timberlake, T. P., & Myers, S. S. (2022). Exploring connections between pollinator health and human health. *Philosophical Transactions of the Royal Society B*, 377(1853), 20210158. https://doi.org/10.1098/rstb.2021.0158

Gemmill-Herren, B. (2016). Pollination services to agriculture - Sustaining and enhancing a key ecosystem service. Food and Agriculture Organization of the United Nations. Routeledge, New York, United States.

Ghazoul, J. (2015). Qualifying pollinators decline evidence. *Science*, 348(6238), 981-982.

Giannini, T. C., Boff, S., Cordeiro, G. D., Cartolano, Jr. E. A., Veiga, A. K., Imperatriz-Fonseca, V. L., & Saraiva, A. M. (2015). Crop pollinators in Brazil: a review of reported interactions. *Apidologie*, 46, 209-223. https://doi.org/10.1007/s13592-014-0316-z

Giannini, T. C., Cordeiro, G. D., Freitas, B. M., Saraiva, A. M., & Imperatriz-Fonseca, V. L. (2015). The dependence of crops for pollinators and the economic value of pollination in Brazil. *Journal of Economic Entomology*, 108(3), 849-857. https://doi.org/10.1093/jee/tov093

Giannini, T. C., Costa, W. F., Cordeiro, G. D., Imperatriz-Fonseca, V. L., Saraiva, A. M., Biesmeijer, J., & Garibaldi, L. A. (2017). Projected climate change threatens pollinators and crop production in Brazil. *PloS One*, 12(8), e0182274. https://doi.org/10.1371/journal.pone.0182274

Goulson, D. (2003). Conserving wild bees for crop pollination. *Journal of Food, Agriculture and Environment*, 1(1), 142-144.

Goulson, D. (2010). Bumblebees: behaviour ecology and conservation. Oxford: Oxford University Press.

Herrmann, J. D., Haddad, N. M., & Levey, D. J. (2018). Mean body size predicts colony performance in the common eastern bumble bee (*Bombus impatiens*). *Ecological Entomology*, 43(4), 458-462. https://doi.org/10.1111/een.12517

Hoehn, P., Tscharntke, T., Tylianakis, J. M., & Steffan-Dewenter, I. (2008). Functional group diversity of bee pollinators increases crop yield. *Proceedings of the Royal Society B*, 275(1648), 2283-2291. https://doi.org/10.1098/rspb.2008.0405

IBGE. (2021). Resultados preliminares do censo agropecuário. (www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/21814-2017-censo-agropecuario.html?edicao=21858&t=resultados).

IPBES. (2016). The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination, and food production. Potts SG, Imperatriz-Fonseca VL, Ngo HT (eds). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn. Germany. 552 pages.

IPBES. (2018). Summary for policymakers of the regional assessment report on biodiversity and ecosystem services for the Americas of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. J. Rice, C.S. Seixas, M.E. Zaccagnini, M. BedoyaGaitán, N. Valderrama, C.B. Anderson, M.T.K. Arroyo, M. Bustamante, J. Cavender-Bares, A. Diaz-de-Leon, S. Fennessy, J. R. García Márquez, K. Garcia, E.H. Helmer, B. Herrera, B. Klatt, J.P. Ometo, V. Rodríguez Osuna, F.R. Scarano, S. Schill and J. S. Farinaci (eds.). IPBES secretariat, Bonn, Germany. 41 pages.

Kevan, P. G. (1997). Honeybees for better apples and much higher yields: study shows pollination services pay dividends. *Canadian Fruit grower*, 14, 16.

Khalifa, S. A. M., Elshafiey, E. H., Shetaia, A. A., El-Wahed, A. A. A., Algethami, A. F., Musharraf, S. G., Alajmi, M. F., Zhao, C., Masry, S. H. D., Abdel-Daim, M. M., M. Halabi, F., Kai, G., Al Naggar, Y., et al. (2021). Overview of bee pollination and its economic value for crop production. *Insects*, 12(8), 688. https://doi.org/10.3390/insects12080688

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E., & Tscharntke, T. (2014). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B*, 281(1775), 24-40. https://doi.org/10.1098/rspb.2013.2440

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B*, 274(1608), 303-313. https://doi.org/10.1098/rspb.2006.3721

Klein, A. M., Boreux, V., Fornoff, F., Mupepele, A. C., & Pufal, G. (2018). Relevance of wild and managed bees for human well-being. *Current Opinion in Insect Science*, 26, 82-88. https://doi.org/10.1016/j.cois.2018.02.011

Knapp, J. L., & Osborne, J. L. (2017). Courgette Production: Pollination demand, supply, and value. *Journal of Economic Entomology*, 110(5), 1973-1979. https://doi.org/10.1093/jee/tox184

Koné, K., Tuo, Y., Yapo, M. L., Traoré, D., Soro, F., & Koua, K. H. (2019). Diversity, abundance and activity of bees in Zucchini (*Cucurbita pepo* L) crops in northern Côte d'Ivoire. *International Journal of Entomology Research*, 4, 41-45.

Krug, C., Alves-Dos-Santos, I., & Cane, J. (2010). Abelhas visitantes de flores de *Cucurbita* (Cucurbitaceae), com ênfase sobre a presença de *Peponapis fervens* Smith (Eucerini - Apidae) - Santa Catarina, sul do Brasil. *Oecologia Australis*, 14, 128-139.

Mascena, V. M., Silva, C. M., Almeida, C. L., Alves, T. T. L., Freitas, B. M. (2018). External activity of colonies of *Melipona quinquefasciata* managed in different types of beehive. *Revista Ciência Agronômica*, 49(4), 683-691. https://doi.org/10.5935/1806-6690.20180077

Malerbo-Souza, D. T., Andrade, M. O., Medeiros, N. M. G., Silva, T. G., & Siqueira, R. A. (2019). Biodiversity and forage behavior of bees in Italian pumpkin crop (*Cucurbita pepo*). *Colloquium Agrariae*, 15(4), 11-21.

Millard, J. W., Freeman, R., & Newbold, T. (2020). Text-analysis reveals taxonomic and geographic disparities in animal pollination literature. *Ecography*, 43(1), 44-59. https://doi.org/10.1111/ecog.04532

Nicodemo, D., Couto, R. H. N., Malheiros, E B., & Jong, D. (2009). Honey bee as an effective pollinating agent of pumpkin. *Scientia Agricola*, 66(4), 476-480. https://doi.org/10.1590/S0103-90162009000400007

Novais, S. M., Nunes, C. A., Santos, N. B., Damico, A. R., Fernandes, G. W., Quesada, M., & Neves, A. C. O. (2016). Effects of a possible pollinator crisis on food crop production in Brazil. *PLoS One*, 11(11), e0167292. https://doi.org/10.1371/journal.pone.0167292

Oldroyd, B. P. (1999). Coevolution while you wait: *Varroa jacobsoni*, a new parasite of western honeybees. *Trends in Ecology and Evolution*, 14(8), 312-315. https://doi.org/10.1016/S0169-5347(99)01613-4

Oliveira-Junior, J. M. B., Santos, L. B. C., Dias-Silva, K. (2022). Ecologia e comportamento de abelhas. Ponta Grossa - PR: Atena, 26 p.

Pannell, J. R. (2017). Plant sex determination. *Current Biology*, 27(5), R191-R197.

Paris, H. S. (2016). Germplasm enhancement of *Cucurbita pepo* (pumpkin, squash, gourd: Cucurbitaceae): progress and challenges. *Euphytica*, 208, 415-438. https://doi.org/10.1007/s10681-015-1605-y

Parra-Tabla, V., Campos-Navarrete, M. J., & Arceo-Gómez, G. (2017). Plant–floral visitor network structure in a smallholder Cucurbitaceae agricultural system in the tropics: implications for the extinction of main floral visitors. *Arthropod-Plant Interactions*, 11, 731-740. https://doi.org/10.1007/s11829-017-9529-1

Peixoto, P. G., Martins, H. L., Pinto, B. C., Franco, A. L., Amaral, L. S., & Castro, C. V. (2022). The significance of pollination for global food production and the guarantee of nutritional security: a literature review. *Environmental Sciences Proceedings*, 15(1), 1-7. https://doi.org/10.3390/environsciproc2022015007

Petersen, J. D., Reiners, S., & Nault, B. A. (2013). Pollination services provided by bees in pumpkin fields supplemented with either *Apis mellifera* or *Bombus impatiens* or not supplemented. *PLoS One*, 8(7), e 69819. https://doi.org/10.1371/journal.pone.0069819

Petersen, J. D., & Nault, B. A. (2014). Landscape diversity moderates the effects of beevisitation frequency to flowers on crop production. *Journal of Applied Ecology*, 51(5), 1347-1356. https://doi.org/10.1111/1365-2664.12287

Petersen, J. D., Huseth, A. S., Nault, B. A. (2014). Evaluating Pollination Deficits in Pumpkin Production in New York. *Environmental Entomology*, 43(5), 1247-1253. https://doi.org/10.1603/EN14085

Phillips, B. W., & Gardiner, M. M. (2015). Use of video surveillance to measure the influences of habitat management and landscape composition on pollinator visitation and pollen deposition in pumpkin (*Cucurbita pepo*) agroecosystems. *PeerJ*, 3, e1342.

Porto, R. G., Cruz-Neto, O., Tabarelli, M., Viana, B. F., Peres, C. A., & Lopes, A. V. (2021). Pollinator-dependent crops in Brazil yield nearly half of nutrients for humans and livestock feed. *Global Food Security*, 31, 100587. https://doi.org/10.1016/j.gfs.2021.100587

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353. https://doi.org/10.1016/j.tree.2010.01.007

Rech, A. R., Agostini, K., Oliveira, P. E., & Machado, I. C. (2014). Biologia da polinização. Rio de Janeiro: Projeto cultural.

Rodrigues, F. A. (2019). Network Centrality: An Introduction. In: Macau, E. (eds) A Mathematical Modeling Approach from Nonlinear Dynamics to Complex Systems. Nonlinear Systems and Complexity, vol 22. Springer, Cham.

Roubik, D. W. (2018). The pollination of cultivated plants: A compendium for practitioners. Food and Agriculture Organization of the United Nations.

Sampson, B. J., Knight, P. R., Cane, J. H., & Spiers, J. M. (2007). Foraging behavior, pollinator effectiveness, and management potential of the new world squash bees *Peponapis pruinosa* and *Xenoglossa strentia* (Apidae: Eucerini). *HortScience*, 42, 459-459.

Shamshiri, R. R., Jones, J. W., Thorp, K. R., Ahmad, D., Man, H. C., & Taheri, S. (2018). Review of optimum temperature, humidity, and vapour pressure deficit for microclimate evaluation and control in greenhouse cultivation of tomato: a review. *International Agrophysics*, 32, 287-302. https://doi.org/10.1515/intag-2017-0005

Shivanna, K. R., Tandon, R., & Kou, M. (2020). 'Global Pollinator Crisis' and Its Impact on Crop Productivity and Sustenance of Plant Diversity. In: Reproductive Ecology of Flowering Plants: Patterns and Processes, eds. R. Tandon, K. Shivanna, and M. Koul, 395–413. Singapore: Springer.

Shuler, R. E., Roulston, T. H., & Farris, G. E. (2005). Farming practices influence wild pollinator populations on squash and pumpkin. *Journal of Economic Entomology*, 98(3), 790-795. https://doi.org/10.1603/0022-0493-98.3.790

Sihag, R. C. (2018). Some unresolved issues of measuring the efficiency of pollinators: experimentally testing and assessing the predictive power of different methods. *International Journal of Ecology*, 2018, 1-13. https://doi.org/10.1155/2018/3904973

Silva, W. P., Gimenes, M. (2014). Pattern of the daily flight activity of *Nannotrigona testaceicornis* (Lepeletier) (Hymenoptera: Apidae) in the Brazilian semiarid region. *Sociobiology*, 61(4), 547-553. https://doi.org/10.13102/sociobiology.v61i4.547-553

Skidmore, A. R., Short, C. A., Dills, C., Goodell, K., & Bessin, R. T. (2019). Preference of *Peponapis pruinosa* (Hymenoptera: Apoidea) for Tilled soils regardless of soil management system. *Environmental Entomology*, 48(4), 961-967. https://doi.org/10.1093/ee/nvz052

Smith, M. R., Singh, G. M., Mozaffarian, D., & Myers, S. S. (2015). Effects of decreases of animal pollinators on human nutrition and global health: a modelling analysis. *The Lancet*, 386(10007), 1964-1972. https://doi.org/10.1016/S0140-6736(15)61085-6

Stokstad, E. (2007). The case of the empty hives. *Science*, 316(5827), 970-972.

Vaissière, B. E., Freitas, B. M., & Gemmill-Herren, B. (2011). Protocol to detect and assess pollination deficits in crops: a handbook for its use. FAO, Roma, Itália.

Valido, A., Rodríguez-Rodríguez, M. C., & Jordano, P. (2019). Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific Reports*, 9, 1-11. https://doi.org/10.1038/s41598-019-41271-5

Velthuis, H. H. W., & van Doorn, A. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie*, 37(4), 421-451. https://doi.org/10.1051/apido:2006019

Vergara, C. H., & Fonseca-Buendía, P. (2012). Pollination of greenhouse tomatoes by the Mexican bumblebee *Bombus ephippiatus* (Hymenoptera: Apidae). *Journal of Pollination Ecology*, 7, 27-30. https://doi.org/10.26786/1920-7603(2012)1

Vidal, M. G., Jong, D., Wien, H. C., & Morse, R. A. (2010). Pollination and fruit set in pumpkin (*Cucurbita pepo*) by honey bees. *Revista Brasileira de Botânica*, 33(1), 106-113.

Waters, S. A., & Taylor, B. H. (2006). Effects of honeybee pollination on pumpkin fruit and seed yield. *HortScience*, 41(2), 370-373. https://doi.org/10.21273/HORTSCI.41.2.370

Wolowski, M., Agostini, K., Rech, A. R., Varassin, I. G., Maués, M., Freitas, L., Saraiva, A. M. (2019). Relatório temático sobre polinização, polinizadores e produção de alimentos no Brasil. Espírito Santo: REBIPP.

FIGURE LEGENDS

Figure 1. Flowchart of selection of pollination studies on zucchini (*Cucurbita pepo* L., Cucurbitaceae) adapted from PRISMA 2020.

Figure 2. Distribution of pollination studies in zucchini (*Cucurbita pepo* L., Cucurbitaceae) and its influence on production. (A) Study sites sampled worldwide. (B) Number of studies conducted over the decades. (C) Number of studies in tropical and temperate areas carried out in different environments. Open: plantations in the field; Closed: greenhouses; Both: both situations.

Figure 3. Distribution of the global literature on zucchinni (*Cucurbita pepo* L., Cucurbitaceae) floral visitors divided into taxonomic groups for the nine main genera and the four main orders. The number of studies performed with each genus is indicated by the fill color in the countries. The "other genera" consist of 28 genera, with the taxonomic orders indicated by fill color, consistent in the panel. The point size represents the frequency of mentioned studies.

Figure 4. Interaction network between pollinators of zucchini (*Cucurbita pepo* L., Cucurbitaceae) and geographical distribution of studies. Circles represent pollinators and squares countries. The thickness of the line is proportional to the number of studies carried out with pollinators in each country.

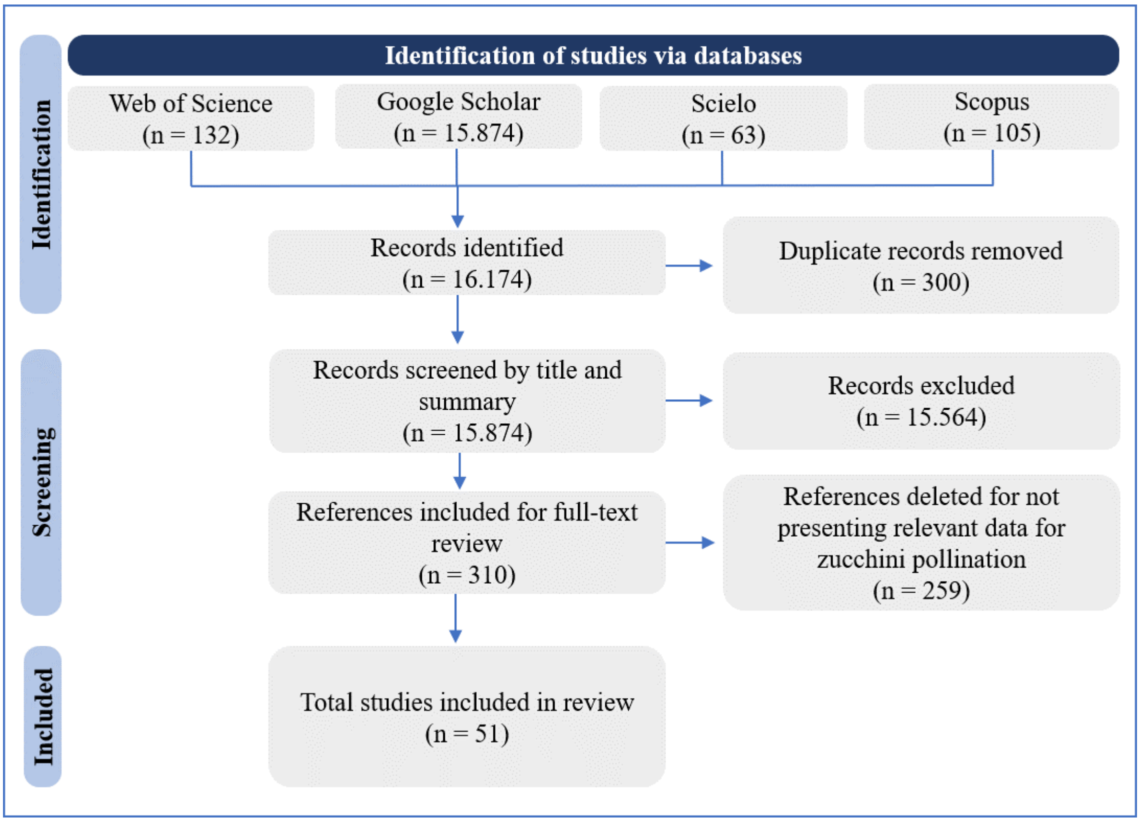

Figure 1. Flowchart of selection of pollination studies on zucchini (*Cucurbita pepo* L., Cucurbitaceae) adapted from PRISMA 2020.

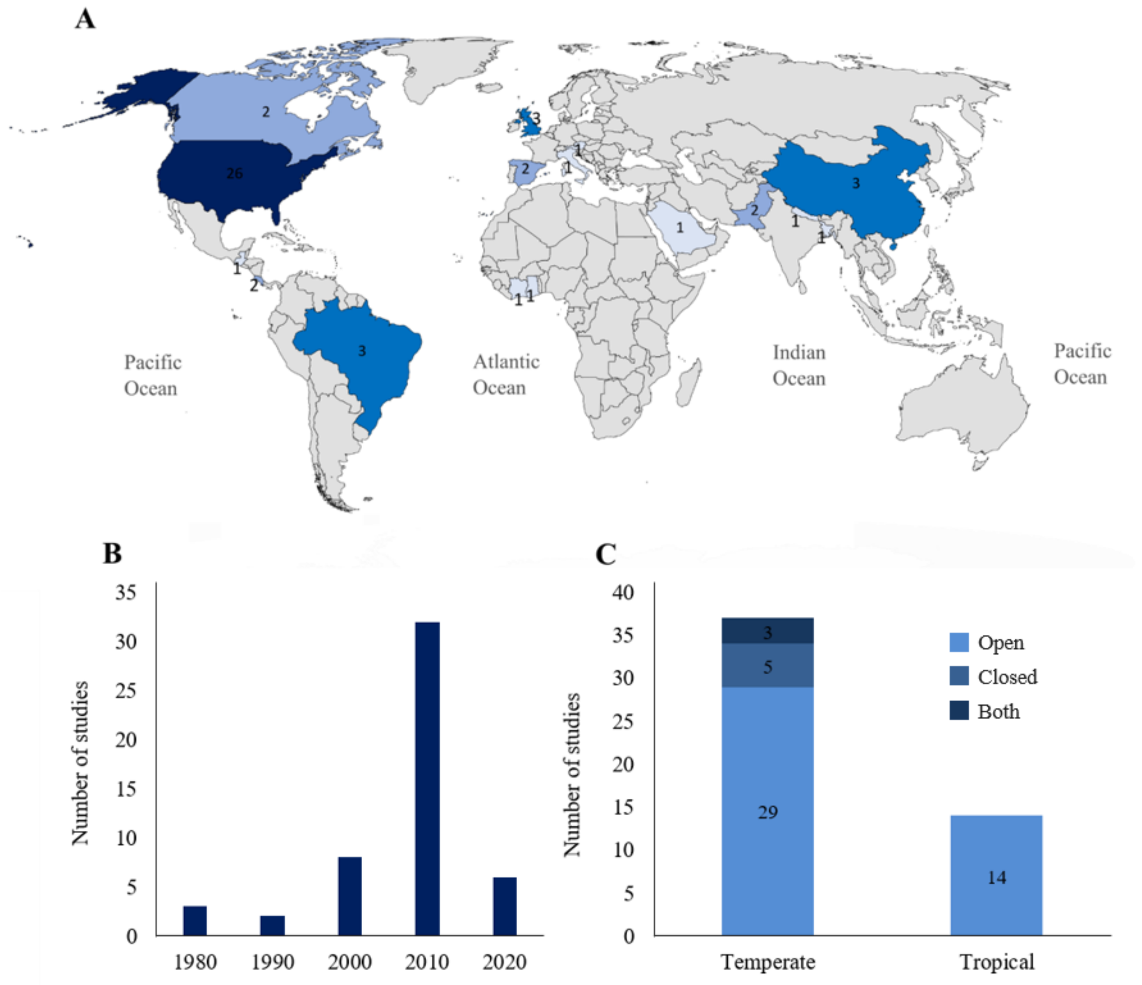

Figure 2. Distribution of pollination studies in zucchini (*Cucurbita pepo* L., Cucurbitaceae) and its influence on production. (A) Study sites sampled worldwide. (B) Number of studies conducted over the decades. (C) Number of studies in tropical and temperate areas carried out in different environments. Open: plantations in the field; Closed: greenhouses; Both: both situations.

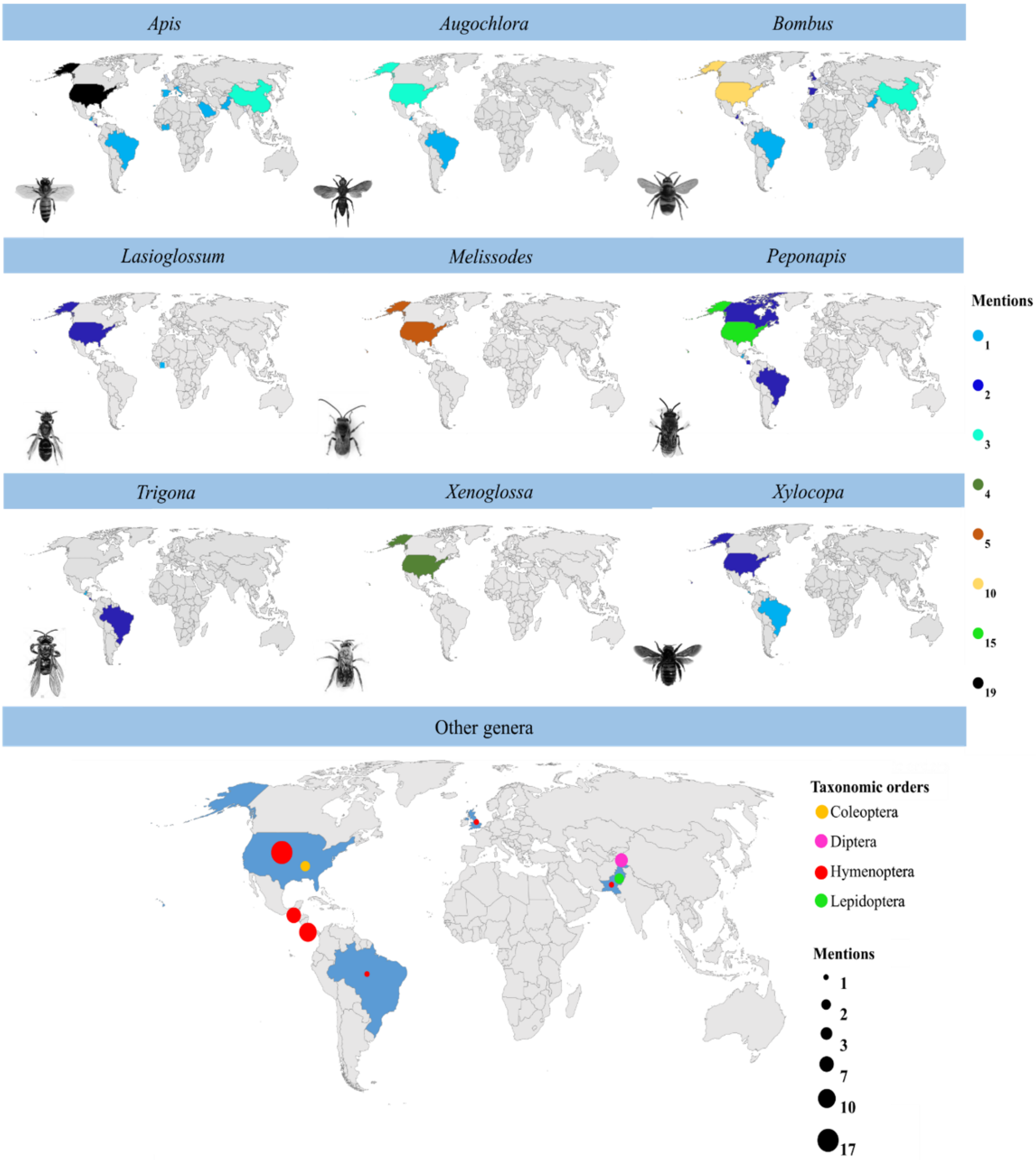

Figure 3. Distribution of the global literature on zucchinni (*Cucurbita pepo* L., Cucurbitaceae) floral visitors divided into taxonomic groups for the nine main genera and the four main orders. The number of studies performed with each genus is indicated by the fill color in the countries. The "other genera" consist of 28 genera, with the taxonomic orders indicated by fill color, consistent in the panel. The point size represents the frequency of mentioned studies.

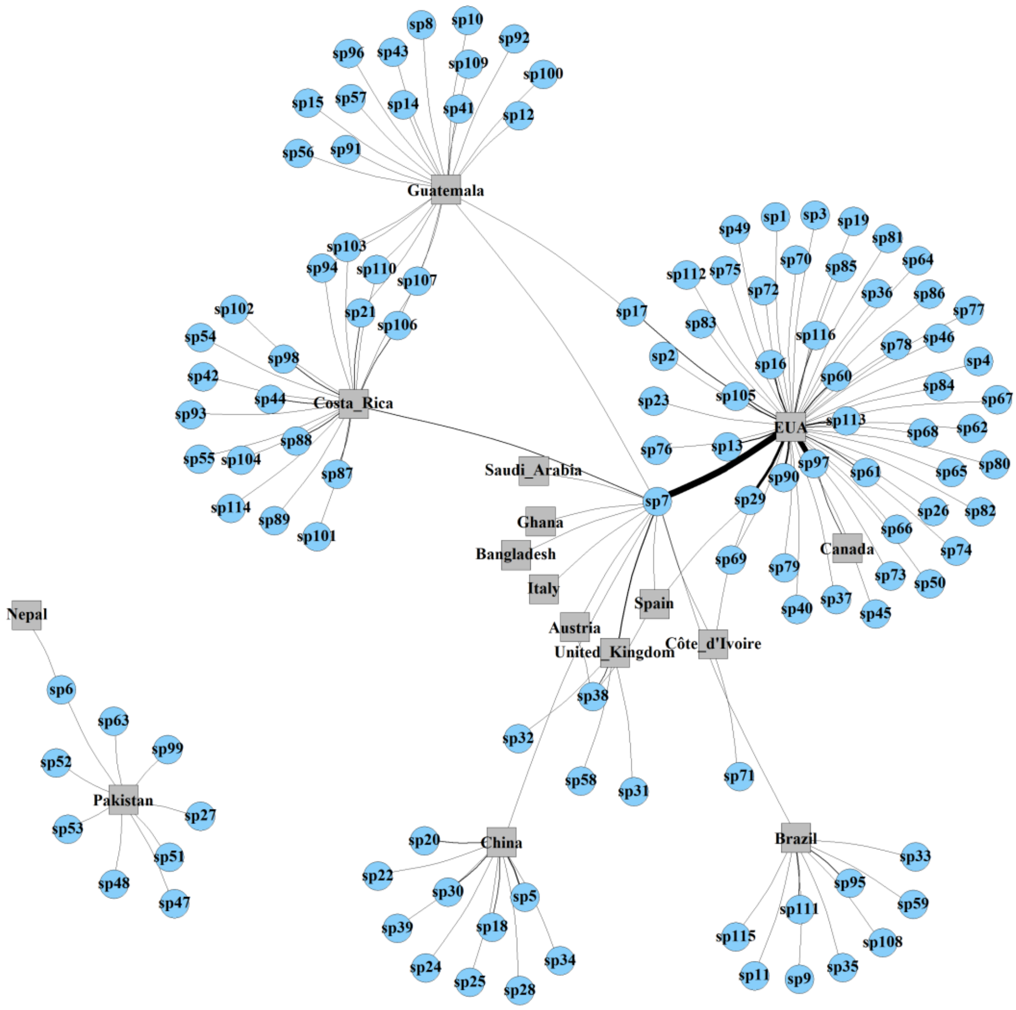

Figure 4. Interaction network between pollinators of zucchini (*Cucurbita pepo* L., Cucurbitaceae) and geographical distribution of studies. Circles represent pollinators and squares countries. The thickness of the line is proportional to the number of studies carried out with pollinators in each country.

CAPÍTULO III

Bee pollination increases fruit set and antioxidant activity in pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae)

Trabalho submetido na revista Scientia Horticulturae em junho de 2024

Bee pollination increases fruit set, weight, and antioxidant activity in pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae)

Isabelle Cristina Santos Magalhães[1]; Gerlayne Teixeira de Souza[1]; Marcelo da Rocha Souza[2]; Liedson Tavares Carneiro[3]; Girlaine Estéfane Cansanção de Almeida[2]; Daniele Silva Ribeiro[2]; Cibele Cardoso de Castro[1,2*]

[1] Programa de Pós-Graduação em Biodiversidade, Universidade Federal Rural de Pernambuco, Rua Manoel de Medeiros s/n, 52171-900, Recife, Pernambuco, Brazil.

[2] Universidade Federal do Agreste de Pernambuco, Avenida Bom Pastor, s/n, 55292272, Garanhuns, Pernambuco, Brazil.

[3] 3Department of Biological Sciences, East Tennessee State University, D.M. Brown Hall, 173 Sherrod Dr, 37614, Johnson City, Tennessee, United States.

*Corresponding author: cibele.castro@ufape.edu.br

Abstract

Due to the global decline of pollinators, global food security is at risk. Crops essentially dependent on pollinators such as pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) are the most affected. Although the importance of pollinators for crop productivity is known, there are many gaps in knowledge regarding the influence of pollination on crop quality, especially on the chemical composition of fruits and seeds. Here, we evaluated the relationship between floral display, frequency of visits and crop production, using three pumpkin plantations in the semi-arid region of NE Brazil as a model. In all sites we recorded floral display, floral visitors, and compared fruit set, fruit quality and seed number between natural (NP) and cross (CP) pollination experiments. Bees were the main pollinators, and *Apis mellifera* and *Trigona spinipes* were the most frequent. Floral display was positively correlated with the total number of visits, which had a positive relation with fruit weight, specifically because of *T. spinipes* visits. Visitation by *A. mellifera* had a negative relationship with pericarp length. Fruit set was about 9% higher in NP than in CP, whose fruits had lower antioxidant activity than the former. Floral display was positively related to the frequency of visits of pumpkin produced in the semi-arid region of northeastern Brazil. Bees were the main pollinators, especially *T. spinipes* and *A. mellifera*, which positively

influenced fruit weight. Pollinators favored fruit set and quality, in addition to promote antioxidant potential.

Keywords: Crop pollination, Apidae, fruit set, cucurbit.

Introduction

Insect pollination is an ecosystem service essential to the production of most agricultural crops worldwide (Klein et al., 2007; Roubik, 2018), generating more than US$500 billion annually (IPBES, 2016). Bees are the main responsible for crop yield, influencing fruit and seed sets and quality (size, weight, and chemical composition (Garibaldi et al., 2013; Klatt et al., 2014; Giannini et al., 2015), as well as seed germination (Kevan & Eisikowitch, 1990). Although the general importance of pollinators for crop productivity is broadly known, there are many gaps of knowledge regarding crop pollination in semiarid tropic environments, as well as the influence of pollination on crop quality, especially chemical composition of fruits and seeds. The influence of animal pollination in the composition of nutritionally and commercially relevant compounds, such as antioxidants, is an emerging area of knowledge that contribute for the management and conservation of pollinators that may improve food security. Antioxidants from fruits and vegetables are beneficial to human health as they prevent oxidative processes, reducing the harmful effects of free radicals on cells that cause the occurrence and spread of degenerative diseases in the body (Gulcin, 2020; Jideani et al., 2021). Furthermore, low concentrations of antioxidants enhance deterioration rates of fruits and vegetables, negatively influencing shelf life (Munteanu & Apetrei, 2021).

Due to the global decline of pollinators, agricultural production is threatened, putting global food security at risk (Potts et al., 2010; Eilers et al., 2011). Crops that are essentially dependent on pollinators are mostly affected (Klein et al., 2018), such as those from the Cucurbitaceae family (Knapp & Osborne, 2019). Cucurbitaceae plants produce unisexual flowers (monoecious), which necessarily require floral visitors to achieve successful fruit and seed production (Delgado-Carrillo et al., 2018; García et al., 2020). In general, *Cucurbita* crops (pumpkins, squash, and some gourds) produce medium to large-sized, showy flowers that offer high volume of nectar to pollinators (Nikolova et al., 2019).

The large amounts of pollen grains produced in male flowers are efficiently transported by insects to the female flowers due to the sticky substances that improve pollen attachment to pollinators' body (Rech et al., 2014). The world main pollinators of *Cucurbita* crops are the bees *Eucera pruinosa* Say, 1867, *Apis mellifera* Linnaeus, 1758 (Chan & Raine, 2021) and *Bombus* species. In Brazil, important pollinators include bees from the families Apidae (*A. mellifera* Linnaeus, 1758, *Melipona* spp., *Trigona spinipes* (Fabricius, 1793)), Adrenidae (*Oxaea flavescens* Klug, 1807, *Bombus morio* (Swederus, 1787)), and beetles from Chrysomelidae family (*Diabrotica* spp.; Giannini et al., 2015). World production of Cucurbita crops is around 35 million tons annually, cultivated on approximately two million hectares, with most production in China and India (FAO, 2024). Pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) is one of the most socioeconomically important vegetables of the genus, with the pollination value in Brazil estimated at R$ 145,173,300.00 (Wolowski et al., 2019).

Considering that Cucurbitaceae crops constitute important components of global agricultural production and are essentially dependent on pollinators, studies that elucidate the relationships between pollination and crop production may be useful for pollinator management and conservation. The objective of this study was to evaluate the relation between floral display, frequency of visits, quantity, and quality of crop production, including fruit antioxidants analyses, using three pumpkin plantations in the semi-arid region of NE Brazil as a model. Our hypotheses are: 1) Floral display (number of flowers per individual) favor frequency of visits, which is positively related to production; 2) Fruit set, quality, and number of seeds are favored by animal pollination.

Materials and methods

Study areas and studied species

The study was carried out at three sites within a semi-arid range located in Pernambuco, NE Brazil (altitude 900m), from January to May 2022. Our study sites comprised two family farming lands (site I: Jucati, 8°40'21" S, 36°27'34" W, and site II: Paranatama, 8°54'50" S, 36°35'34" W) and the experimental research field of the Universidade Federal do Agreste de Pernambuco (site III: Garanhuns, 8°90'64" S, 36°49'43" W). The climate of the Garanhuns region is the As' type (warm and wet, according to Köppen's classification). The average annual precipitation, humidity and temperature are 782,5 mm, 82.5 % and 21.2 °C, respectively (APAC, 2023). The climate of the regions of sites I and II is type BSh (semi-arid climate of low latitudes and altitudes, according to Köppen's classification). The average annual precipitation and temperature are 641,5 / 550,3 mm and 28 °C, respectively (APAC, 2023; CPTEC/INPE, 2024). The dominant soil types in the region are ferric Oxisols or Argisols.

Cucurbita moschata is a monoecious crop, i.e., has unisexual flowers, with temporal separation between male and female flowers within a plant (proterandry; Agbagwa et al., 2007). At each site, 64 pumpkin seedlings (*C. moschata*, cultivar 'Jacarezinho') obtained from commercial seeds were planted with a row spacing of 3×1 m, following regular technical recommendations (IPA, 2008). We applied organic manure to soil, with no other fertilizers or pesticides, following the same protocol as the small farmers. Irrigation was carried out with drippers spaced approximately 50 cm apart.

Evaluation of floral biology components

Although the floral biology of pumpkins has been well-investigated (Agbagwa et al., 2007; Lima et al., 2022), there may be variations among cultivars that can influence pollination outcomes (Klein et al., 2007). Thus, we described the floral longevity, evaluated the stigma receptivity, and searched for presence of scent glands. For floral longevity, we monitored 10 male and 10 female flowers from different individuals from opening to senescence. For stigma receptivity, we tested for peroxidase activity in 10 female flowers distributed in 10 individuals hourly between 05h00 and 11h00 (Dafni et al., 2005). To detect scent glands on flowers, we immersed 10 flowers in a neutral red solution for 10 min (Dafni et al., 2005). In this procedure, any staining indicates the presence of scent glands.

Flower visitation and pollinator survey

Flower visitors were recorded during 56 h of observations (around 19 h per area). In each area, 25 plants were randomly selected and observed in a circuit where the observer remained 15 min in each plant, between 6h00 and 10h00 during three non-consecutive days under favorable weather conditions. During observations, floral visitor morphospecies were recorded, as well as the number of flowers visited by each morphospecies in the plant. All observed plants had the total number of flowers recorded (floral display). Flower visitors were collected after observations and identified by specialists.

Pollination experiment

To understand the influence of the pollination service provided by the flower visit insects on the quantity and quality of pumpkin production, we set two treatments: natural pollination (NP) and cross-pollination (CP). For each treatment, we used 50 flowers from 50 individuals at each site. For NP, flowers were marked and left accessible to flower visitors. For CP, flowers were bagged in pre-anthesis and then cross-pollinated with pollen from three different individuals. After hand crosses, the flowers were maintained bagged until senescence. Forty days after anthesis, the number of fruits resulting from the experiments was counted and fruits were harvested, and the following commercially relevant traits were recorded: Seed number, fruit weight (g), pericarp (mm), pH and antioxidants.

Each trait was measured in all harvested fruits. Fruit weight was quantified using a digital scale, whilst pericarp length was measured with a digital caliper. pH was determined with a calibrated pH meter (AOAC, 1992).

The evaluation of antioxidant activity was carried out as these compounds such as phenolics, vitamin C, tocopherols, carotenoids, and flavonoids have nutritional importance such as oxidative deterioration, reducing the risk of developing various disorders caused by oxidative stress (Gulcin, 2020). The ABTS method (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) was used in ten fruits of each treatment from Paranatama. A sample of 5g of each fruit was added in 20 mL of 80% aqueous methanol solution, followed by grinding in a mixer, shaking on a shaking table for 60 minutes and centrifugation at 15,000 rpm for 15 min. The supernatant was collected and filtered in filter paper, transferred to a 50 mL volumetric flask, and made up to volume with 80% methanol (modified from Amariz, 2011; Kulczyński et al., 2020). Antioxidant activity by ABTS was performed according to the modified methodology of Singh et al. (2016) described below. The ABTS radical stock solution was produced by reacting the ABTS solution (25 mL) with potassium persulfate (400 µL) and left to react in the dark at room temperature for 16 h. The ABTS solution was then diluted in pure ethyl alcohol until it reached an absorbance of 0.700 ± 0.05 at 734 nm. A 0.5 mL aliquot of the sample was added to a falcon tube containing 3.5 mL of the free radical ABTS, left protected from light for six minutes and the absorbance was measured in triplicate. Trolox standard solution was used to perform the calibration curve, and the results were expressed as µmol Trolox/L.

Data analysis

Influence of pollination on production

To test differences in the fruit set, number of seeds and fruit characteristics between NP and CP, generalized linear mixed models (GLMM) were generated, therefore considering treatment as a fixed effect, and studied planting and plant identity as random effects. For fruit set (n = 287), a binomial model was adjusted, including fruit formation or non-formation as a response variable using the *glmer* function from the lme4 package. For the other variables, only the portion of the sample that produced fruit (n = 232) and models adjusted according to their nature were used. Thus, considering the number of seeds per fruit as the response variable, a Poisson model was adjusted, but to control overdispersion, the use of a model with negative binomial error distribution was more appropriate using the *glmer.nb* function (*lme4 package*). For fruit traits, the number of seeds was also included as a fixed effect along with treatment, since the quantity of seeds can influence fruit quality (Santos et al., 2021). Gaussian models were fitted for fruit weight, pericarp and pH using the *lmer* function (lme4 package). The interaction between seed number and treatment was never significant and was therefore not included in the models. To compare antioxidants (πmol Eq. a Trolox/L ext), a Gaussian model was generated including only plant identity as a random effect, since the test was carried out on a single planting. For all models, the significance of fixed effects was tested using the *Anova* function from the *car* package.

Frequency of visits at production and floral display

As the total number of floral visits was represented by 67% of visits by *T. spinipes* and 27% by *A. mellifera* (the remaining 6% was distributed among five other species), we evaluated whether the quantity and quality of production are influenced by the number of visits flowers received by the two bees. For this, two sets of models were built: (1) one considering the influence of the total number of visits on the production variables and (2) the other including visitation by *A. mellifera* and *T. spinipes* as fixed effects in GLMMs, incorporating studied planting and plant identity as random effects. The interaction between *A. mellifera* and *T. spinipes* visitation was included in the models to test the joint effect of both species on production. For fruit set (n = 126), a model with binomial distribution (*glmer*) was adjusted again, including fruit formation and non-formation as a response variable, with the other variables (seed number, fruit weight, pericarp length and pH) being analyzed with the portion of the samples that produced fruit (n = 114).

For seed number, models with negative binomial distribution (*glmer.nb*) were fitted and for pH, Gaussian models were fitted, as previously described. However, for fruit weight and pericarp length, models with log-normal distribution were adjusted to achieve the assumption of normality of model residuals. To test the significance of the models, the *Anova* function from the car package was also used.

To test whether the floral display influences the number of total visits by *A. mellifera and T. spinipes*, GLMMs were fitted with the same random effects structure, considering the number of flowers per plant at the time of observations as a fixed effect. As the three models suffered from overdispersion when fitted to the Poisson distribution, models with a negative binomial distribution were used (*glmer.nb*). The significance of the parameters was tested using the procedure described in the previous analyses. All analyzes described above were conducted in R v.4.2.3 (R Core Team, 2023).

Results

The plants are protandrous, i.e., the anthesis of male flowers occurs two weeks before the female flowers. The ratio of male and female flowers was 8:1. Floral longevity lasted from 5:30 am to 11:00 am. The stigma remained receptive from the bud stage until senescence, and, in male flowers, pollen was available from flower opening. Scent glands were detected on the corolla and anthers tissues. Both male and female flowers are nectar-rewarding.

Visits occurred between 6:00 am and 11:00 am, with a peak between 7:00 am and 9:00 am in all sites (Fig. 1). Seven species were recorded visiting the flowers, distributed in the three sites (Table 1). A total of 447 visits from three bee (*Apis mellifera*, *Trigona spinipes* and *Xylocopa* (*Neoxylocopa*)) and a wasp species (*Polybia* sp.) were recorded in Jucati, 496 visits from six bee species and *Polybia sp.* in Paranatama and 181 bee species and *Polybia* sp. in Garanhuns (Table 1). The most frequent floral visitors were by far *Apis mellifera* and the stingless bee *Trigona spinipes* (Table 1, Fig. 1).

All floral visitors behaved as pollinators since they contacted anthers and stigma during visits. All bee species foraged for pollen and nectar on male flowers, and nectar on female ones; *Polybia* sp. collected only nectar from male and female flowers. The bees *Augochlora* sp., *Paratrigona* cf. *incerta* and *Xylocopa* (*Neoxylocopa*) sp., as well as *Polybia* sp, had low frequency of visits, thus being considered as occasional pollinators. Generally, *T. spinipes* foraged in groups, being aggressive towards other bees that tried to land on the flower.

The mean number of flowers per individual was 6.45 ± 3.21. Floral display was positively correlated with the total number of visits (χ^2 = 12.28, *df* = 1, $p < 0.001$), specifically with the number of *A. mellifera* visits (χ^2 = 14.29, *df* = 1, *p* < 0.001), but not with the number of visits by *T. spinipes* (χ^2 = 0.01, *df* = 1, *p* = 0.912; Fig. 2).

The total number of visits had a significant influence on fruit weight, what was specifically attributed to the positive impact of visits by *T. spinipes* (Table 2, Fig. 3). On the other hand, the total number of visits did not explain fruit set, pericarp length, pH, and seed number (Table 2). There was no influence of the number of visits by *A. mellifera* or both *A. mellifera* and *T. spinipes* combined on fruit weight (Table 2). Visitation by *A. mellifera* had a negative relationship with pericarp length (Table 2, Fig. 3).

Fruit set was about 9% higher in NP than in CP, whose fruits had lower antioxidant activity than the former (Table 3, Fig. 4). There was no effect of treatments on the other parameters evaluated (fruit weight, pericarp length, pH, and seed number; Table 3). Seed number was positively related to fruit weight (Table 3, Fig. 5), but did not influence pericarp length or pH (Table 3).

Discussion

Floral display of pumpkin plants grown in the family farming orchards was positively related to frequency of visits of the main pollinators (the alien feral Africanized honeybees and *T. spinipes*). Pollination services positively influenced fruit set, weigh, and antioxidant potential. Visitation by *T. spinipes* had a positive impact on production, whereas visitation by *A. mellifera* was related to lower pericarp width.

The floral biology observed here corroborate other pumpkin cultivars from previously published studies (Agbagwa et al., 2007; Lima et al., 2022). The positive impact of the massive flowering on the attractiveness of pollinators resulted both from visual and olfactive clues, as well as the high availability of floral resources (pollen and nectar; Hovestadt et al., 2018; Albuquerque-Lima et al., 2020). In this way, there may be an increase in the density of pollinators on a landscape scale, benefiting other crops (Fijen et al., 2019). In agricultural crops, crops with mass flowers are often preferred by floral visitors, resulting in high visitation rates and increased production yield (Pufal et al., 2017).

The higher fruit set and antioxidant activity resulted from NP when compared to CP is possibly due to the greater genetic diversity of the pollen loads deposited in NP. Bees tend to visit flowers from many individuals, whereas flowers from only three individuals were used in the CP experiment. This better performance of NP reinforces the importance of pollinators’ frequency of visits and diversity for fruit production. The high functional diversity provided by distinct morphologies and behaviors of pollinators is known to improve crop yield (Garibaldi et al. 2013, 2016; Fijen et al. 2019) and quality (Kamo et al., 2022; Chai et al., 2023). The importance of pollinators for global agricultural production lies especially in this relationship, which is even more relevant for crops that are essentially dependent on pollinators, such as pumpkin (Wolowski et al., 2019).

The relation between pollen loads deposited onto stigmas and quality aspects of crop production (dimensions and chemical composition) is called xenia (Chai et al., 2023 and references therein). It is defined as the chemical influence of tissues bearing paternal genes upon maternal tissues (Denney 1992), being recorded in fruits (e.g., Gaaliche 2011; Sabir et al., 2015; Gharaghani, 2017; Chai et al., 2023), grasses (Pozzi et al., 2018) and vegetables (Piotto et al., 2013). Pollen, pollen tubes and fertilized ovules diffuse growth regulatory substances (auxins, gibberellins, brassinosteroids, cytokinins, polyamines, ethylene, and others) across ovary tissues that develop into fruit and seed (Perazza et al., 1998), regulating seed development and fruit size (Ozga & Reinecke, 2003, Balaguera-López et al., 2020). This physiological process is not well understood, as pollinators can influence agricultural production in different ways, from not interfering in production (as in beans, Paulino et al. 2023), to improving quantity and quality (as in strawberries, Abrol et al., 2019). Similarly, the characteristics that each pollinator influences can vary, as noted here. Although the total number of visits had a significant influence on fruit weight, it did not explain the other attributes (fruit set, pericarp length, pH nor seed number). Also, the number of visits by *A. mellifera* and of the joint action with *T. spinipes* did not influence fruit weight, but visitation by *A. mellifera* decreased pericarp length.

The positive relation between number of seeds and fruit weight may also be explained by xenia, since a high number of fertilized ovules implies a greater production and diffusion of regulatory substances. The positive relation between seed number and fruit size is well known (Delaplane & Mayer, 2000; McGrady et al., 2020; Petersen et al., 2013). It is important to highlight, however, that fruit and seed dimensions and chemical composition may also be related to aspects other than pollination, such as nutrient and water availability, as well as climatic conditions (Schlering et al., 2020).

The positive influence of pollination on the production of antioxidant compounds observed here was also recorded in other studies, such as in coffee (Canzi et al., 2023), pistachio (Sharifkhah et al., 2020), and perrilla (*Perilla frutescens*, Lamiaceae Ferrazzi et al., 2017). Vegetables from the Cucurbitaceae have antioxidant substances of high biological importance, and important in the use of phytochemicals and herbal medicines (Akhter et al., 2022). Studies carried out with the ABTS+ radical showed that species such as *C. pepo* (Kulczyński et al., 2020), *C. maxima*, *Citrullus lanatus* and *Cucumis melo* (Singh et al., 2016) have high antioxidant capacity in both /fruit pulp and peel. This is the first report on the influence of pollination on antioxidants od Cucurbitaceae crops so far.

The positive influence *T. spinipes* pollination on fruit weight may be explained by its behavior, as individuals walked for a longer time on the flower sexual organs, contacting pollen and stigma more frequently than other species. This bee species is often considered to only cause damage to flowers and fruits (Oda & Oda, 2007), and to act as nectar thieve, making holes in the base of the calyx of many plants of economic interest such as *Crotalaria vitellina* (Bergamo & Sazima, 2018), *Jacaranda rugosa* (Milet-Pinheiro & Schlindwein, 2009) and *Cucurbita moschata* (Serra & Campos, 2018). However, it is also frequently observed pollinating agricultural crops of Cucurbitaceae (chayote -*Sechium edule*; Malerbo-Souza et al., 2023; zucchini - *Cucurbita pepo*; Malerbo-Souza et al., 2019; gourds-Cucurbita spp., McGrady et al., 2019), Fabaceae (Paulino et al., 2021, Paulino et al., 2023), Rosaceae (strawberry - *Fragaria x ananassa*, MacInnis & Forrest, 2019), Rutaceae (tangelos - *Citrus clementina* hort. × (*C. paradisi* Macfad.) × *C. reticulata* Blanco)], Santos et al., 2021), Proteaceae (macadamia - *Macadamia integrifolia*, Santos et al., 2020, Solanaceae (eggplant-*Solanum melongena*, Campos et al., 2022; tomato - *Solanum lycopersicum*,Vinícius-Silva et al., 2017), and Vitaceae (grape - *Vitis labrusca*, Baronio et al., 2021). In natural ecosystems it also contributes to pollination process (Silva et al., 2021). For example, although it damages 96% of *Nymphaea pulchella* flowers through direct and indirect florivory, it allows pollination to occur due to direct collection of large quantities of pollen from the anthers (Chalegre et al., 2020).

The frequent occurrence of T. *spinipes* is probably due to its larger populations when compared to most bees in open environments (Valadares et al., 2023). It has aggressive behavior that prevents other species of bees from reaching flowers, monopolizing floral resources (Nieh et al., 2004, 2005). Thus, it is reasonable to affirm that *T. spinipes* plays a relevant pollination service in agriculture. However, the cost-benefit varies with plant species, environment and season. In our study, floral display did not explain *T. spinipes* visitation, corroborating its tendency to visit the crop independently of the number of flowers and, in the case of *C. moschata*, improving fruit production. On the other hand, a higher floral display promotes *A. mellifera* pollination, which negatively influences pericarp length.

Although *A. mellifera* is a generalist species widely managed around the world, both for honey production and crop pollination, several studies have recorded that it ecologically displaces native bees and reduces pollination (Potts et al., 2010, Valido et al., 2019), and even the fitness of the progeny of native plants (Page & Williams, 2023).

According to our results, the need for management actions implemented by farmers to conserve native bees in agricultural crops is clear, such as the preservation of native vegetation around crops and the installation of agroforestry systems to benefit the diversity of native bees.

Conclusions

Floral display was positively related to the frequency of visits of pumpkin produced in the semi-arid region of northeastern Brazil. Bees were the main pollinators, especially *T. spinipes* and *A. mellifera*, which positively influenced fruit weight. Pollinators favored fruit set and quality, in addition to promote antioxidant potential.

Competing Interests

Authors have no competing interests.

Funding

ICSM and GTS received financial support from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Financing Code 001), MRS from Fundação de Amparo à Ciência e Tecnologia de Pernambuco (BIC-0700-2.03/21), and CCC from Conselho Nacional de Desenvolvimento Científico e Tecnológico (research grant: 428303/2018-8, fellowship: 312372/2021-3).

Aknowledgements

We thank the Programa de Pós-Graduação em Biodiversidade of the Universidade Federal Rural de Pernambuco and the Universidade Federal do Agreste de Pernambuco for the institutional support. We also thank the farmers for logistical support.

Author Contribution

ICSM, MRS and GTS carried out the field research.

LTC coordinated the experimental design and data analyses.

GECA and DSR coordinated the antioxidant analysis.

CCC coordinated the study and obtained funding.

All authors wrote the manuscript.

All authors read and approved the manuscript.

References

Abrol, D. P., Gorka, A. K., Ansari, M. J., Al-Ghamdi, A., Al-Kahtani, S. (2019). Impact of insect pollinators on yield and fruit quality of strawberry. *Saudi Journal of Biological Sciences*, 26(3), 524-530. https://doi.org/10.1016/j.sjbs.2017.08.003

Agbagwa, I. O., Ndukwu, B. C., Mensah, S. I. (2007). Floral Biology, Breeding System, and Pollination Ecology of *Cucurbita moschata* (Duch. ex Lam) Duch. ex Poir. Varieties (Cucurbitaceae) from Parts of the Niger Delta, Nigeria. *Turkish Journal of Botany*, 31, 451-458.

Akhter, K., Bibi, A., Rasheed, A., Rehman, Su, Shafique, U., Habib, T. (2022). Indigenous vegetables of family Cucurbitaceae of Azad Kashmir: A key emphasis on their pharmacological potential. *PLoS ONE*, 17(6), e0269444. https://doi.org/10.1371/journal.pone.0269444

Albuquerque-Lima, S., Domingos-Melo, A., Nadia, T. C. L., Bezerra, E. L. S., Navarro, D. M. A. F. Milet-Pinheiro, P., Machado, I. C. (2020). An explosion of perfume: Mass flowering and sphingophily in the Caatinga dry region in Brazil. *Plant Species Biology*, 35(3), 1–13. https://doi.org/10.1111/1442-1984.12277

APAC. (2023). Atlas climatológico do Estado de Pernambuco: normais climatológicas 1991-2020. Agência Pernambucana de Águas e Clima. Gerência de Meteorologia e Mudanças Climáticas. – Recife: APAC, GMMC, 148p., 2023.

Azpiazu, C., Medina, P., Adán, Á., Sánchez-Ramos, I., Estal, P. d, Fereres, A., Viñuela, E. (2020). The Role of Annual Flowering Plant Strips on a Melon Crop in Central Spain. Influence on Pollinators and Crop. *Insects*, 11(1), 66. https://doi.org/10.3390/insects11010066

Balaguera-López, H. E., Fischer, G., Magnitskiy, S. (2020). Seed-fruit relationships in fleshy fruit: Role of hormones. A review. *Revista Colombiana de Ciencias Hortícolas*, 14(1), 90-103. https://doi.org/10.17584/rcch.2020v14i1.10921

Baronio, G. J., Souza, C. S., Silva, N. N. A., Moura, N. P., Leite, A.V., Santos, A. M. M., Maciel, M. I. S., & Castro, C. C. (2021). Different visitation frequencies of native and non-native bees to vines: how much vegetation is necessary to improve fruit production? *Plant Biology*, 23, 923-930. https://doi.org/10.1111/plb.13327

Bergamo, P. J., Sazima, M. (2018). Differential outcomes of nectar robbing on the reproductive success of a melittophilous and an ornithophilous species. *International Journal of Plant Sciences*, 179(3), 000–000. https://doi.org/10.1086/696234

Campos, G. P. A., Barros, C. T., Carneiro, L. T., Santa-Martinez, E., Milfont, M. O., Castro, C. C. (2022). Pollinator efficiency in openly grown eggplants: can non-vibrating bees produce high-quality fruits? *Arthropod-Plant Interactions,* 16, 159–170. https://doi.org/10.1007/s11829-022-09885-1

Canzi, F. A., Meireles, D. A. L., Valdez, A. S. B., Abrantes, L. S., Boroski, M., Augusto, S. C., Valderrama, P., Toci, A. T. (2023). Effect of pollination on the composition of raw arabica coffee (*Coffea arabica* L.): antioxidant capacity, bioactive compounds, and volatiles precursors. *Journal of the Science of Food and Agriculture*, 103, 5578-5587. https://doi.org/10.1002/jsfa.12635

Chai, Y., Hong, W., Liu, H., Shi, X., Liu, Y., Liu, Z. (2023). The pollen donor affects seed development, taste, and flavor quality in 'hayward' kiwifruit. *International Journal of Molecular Sciences*, 24(10), 8876.

Chalegre, S. L., Domingos-Melo, A., Lima, C. T., Giulietti, A. M., Machado, I. C. (2020). *Nymphaea pulchella* (Nymphaeaceae) and *Trigona spinipes* (Apidae) interaction: From florivory to effective pollination in ponds surrounded by pasture. *Aquatic Botany*, 166, 103267. https://doi.org/10.1016/j.aquabot.2020.103267

Chan, D. S. W., Raine, N. E. (2021). Hoary Squash Bees (*Eucera pruinosa*: Hymenoptera: Apidae) Provide Abundant and Reliable Pollination Services to *Cucurbita* Crops in Ontario (Canada). *Environmental Entomology*, 50, 968-981. https://doi.org/10.1093/ee/nvab045

CPTEC/INPE. (2024). Centro de Previsão de Tempo e Estudos Climáticos. Instituto Nacional de Pesquisas Espaciais. (https://tempo.cptec.inpe.br/pe)

Dafni, A., Kevan, P. G., Husband, B. C. (2005) Practical pollination biology. Enviroquest Ltd, Cambridge.

Delaplane, K.S. and D.F. Mayer. (2000). Crop pollination by bees. CAB Intl., Wallingford, U.K.

Delgado-Carrillo, O., Martén-Rodríguez, S., Ashworth, L., Aguilar, R., Lopezaraiza-Mikel, M., Quesada, M. (2018). Temporal variation in pollination services to *Cucurbita moschata* is determined by bee gender and diversity. *Ecosphere*, 9(11), e02506. https://doi.org/10.1002/ecs2.2506

Eilers, E. J., Kremen, C., Greenleaf, S. S, Garber, A. K., Klein, A. M. (2011) Contribution of pollinator-mediated crops to nutrients in the human food supply. *PLoS ONE*, 6, e21363. https://doi.org/10.1371/journal.pone.0021363

Ferrazzi, P., Vercelli, M., Chakir, A., Romane, A., Mattana, M., Consonni, R. (2017). Pollination effects on antioxidant content of *Perilla frutescens* seeds analysed by NMR spectroscopy. *Natural Product Research*, 31, 2705-2711. https://doi.org/10.1080/14786419.2017.1292267

Fijen, T. P. M., Scheper, J. A., Boekelo, B., Raemakers, I., Kleijn, D. (2019). Effects of landscape complexity on pollinators are moderated by pollinators' association with mass-flowering crops. *Proceedings of the Royal Society B: Biological Sciences*, 286(1900), 20190387. https://doi.org/10.1098/rspb.2019.0387

García, A., Aguado, E., Garrido, D., Martínez, C., Jamilena, M. (2020). Two androecious mutations reveal the crucial role of ethylene receptors in the initiation of female flower development in *Cucurbita pepo*. *The Plant Journal*, 103(4), 1548-1560. https://doi.org/10.1111/tpj.14846

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A. et al. (2013) Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, 339(6127), 1608–1611. https://doi.org/10.1126/science.123020

Garibaldi, L. A., Carvalheiro, L. G., Vaissière, B. E., Gemmill-Herren, B., Hipolito, J., Freitas, B. M. et al. (2016). Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science* 351:388–391. https://doi.org/10.1126/science.aac7287

Giannini, T. C., Boff, S., Cordeiro, G. D., Cartolano, E. A., Veiga, A. K., Imperatriz-Fonseca, V. L., Saraiva, A. M. (2015). Crop pollinators in Brazil: a review of reported interactions. *Apidologie*, 46(2), 209–223. https://doi.org/10.1007/s13592-014-0316-z

Gulcin, I. (2020). Antioxidants and antioxidant methods: an updated overview. *Archives of Toxicology*, 94, 651–715. https://doi.org/10.1007/s00204-020-02689-3

Hovestadt, T., Mitesser, O., Poethke, A., Holzschuh, A. (2018). Explaining the variability in the response of annual eusocial insects to mass-flowering events. *Journal of Animal Ecology*, 88(1). https://doi.org/10.1111/1365-2656.12908

IPBES. (2016). The assessment report of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services on pollinators, pollination, and food production. Potts SG, Imperatriz-Fonseca VL, Ngo HT (eds). Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn. Germany. 552 pages.

IPA (Instituto de Pesquisas Agropecuárias) (2008). Recomendações de adubação para o Estado de Pernambuco, 2nd edn. IPA, Recife PE.

Kamo, T., Nikkeshi, A., Tawaratsumida, T., Tanaka, Y., Nakamura, S., Kishi, S. (2022). Pollination efciency of bumblebee, honeybee, and hawkmoth in kabocha squash, *Cucurbita maxima*, production in Kagoshima, Japan. *Applied Entomology and Zoology* 57, 119–129. https://doi.org/10.1007/s13355-021-00766-2

Kevan, G., Eisikowitch, D. (1990). The effects of insect pollination on canola (*Brassica napus* L. cv. O.A.C. Triton) of seed germination. *Euphytica*, 45, 39-41. https://doi.org/10.1007/BF00032148

Klatt, B. K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E. et al. (2014). Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B: Biological Sciences*, 281, 24–40. https://doi.org/10.1098/rspb.2013.2440

Klein, A. M., Boreux, V., Fornoff, F., Mupepele, A. C., Pufal, G. (2018). Relevance of wild and managed bees for human well-being. *Current Opinion in Insect Science*, 26, 82-88. https://doi.org/10.1016/j.cois.2018.02.011

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B*, 274(1608), 303-313. https://doi.org/10.1098/rspb.2006.3721

Knapp, J. L., Osborne, J. L. (2019). Cucurbits as a model system for crop pollination management. *Journal of Pollination Ecology*, 25(9), 89-102. https://doi.org/10.26786/1920-7603(2019)535

Kulczyński, B., Sidor, A., Gramza-Michałowska, A. (2020) Antioxidant potential of phytochemicals in pumpkin varieties belonging to *Cucurbita moschata* and *Cucurbita pepo* species. *CyTA - Journal of Food*, 18(1), 472-484, https://doi.org/10.1080/19476337.2020.1778092

Jideani, A. I. O., Silungwe, H., Takalani, T., Omolola, A. O., Udeh, H. O., Anyasi, T. A. (2021). Antioxidant-rich natural fruit and vegetable products and human health. *International Journal of Food Properties*, 24(1), 41-67. https://doi.org/10.1080/10942912.2020.1866597

Lima, M. V., Oliveira, F. I. C., Ramos, S. R. R., Freitas, B. M., Aragão, F. A. S. (2022). Flowering phenology and floral biology in pumpkin cultivars. *Revista Ciência Agronômica*, 53, e20218013. https://doi.org/10.5935/1806-6690.20220024

Malerbo-Souza, D. T., Costa, C. F. S., Pimentel, A. C. S., Andrade, M. O., Siqueira, R. A., Silva, R. C. B., Souza, C. M., Souza, F. G. (2023). Stingless bee *Trigona spinipes* (Hymenoptera: Apidae) behavior on chayote flowers (*Sechium edule*). *Acta Scientiarum. Animal Sciences*, 45, e56760. https://doi.org/10.4025/actascianimsci.v45i1.56760

Malerbo-Souza, D. T., Andrade, M. O., Medeiros, N. M. G., Silva, T. G., Siqueira, R. A. (2019). Biodiversity and forage behavior of bees in Italian pumpkin crop (*Cucurbita pepo*). *Colloquium Agrariae*, 15(4), 11-21. https://doi.org/10.5747/ca.2019.v15.n4.a307

MacInnis, G., Forrest, J. R. K. (2019). Pollination by wild bees yields larger strawberries than pollination by honey bees. *Journal of Applied Ecology*, 56, 824-832. https://doi.org/10.1111/1365-2664.13344

McGrady, C. M., Troyer, R., Fleischer, S. J. (2020). Wild Bee Visitation Rates Exceed Pollination Thresholds in Commercial *Cucurbita* Agroecosystems. *Journal of Economic Entomology*, 113(2), 562–574. https://doi.org/10.1093/jee/toz295

Milet-Pinheiro, P., Schlindwein, C. (2009). Pollination in *Jacaranda rugosa* (Bignoniaceae): euglossine pollinators, nectar robbers and low fruit set. *Plant Biology*, 11, 131–141. https://doi.org/10.1111/j.1438-8677.2008.00118.x

Munteanu, I. G., Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A Review. *International Journal of Molecular Sciences*, 22(7), 3380. https://doi.org/10.3390/ijms22073380

Nieh, J. C., Barreto, L. S., Contrera, F. A. L., Imperatriz-Fonseca, V. L. (2004). Olfactory eavesdropping by a competitively foraging stingless bee, *Trigona spinipes*. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1548), 1633–1640. https://doi.org/10.1098/rspb.2004. 2717

Nieh, J. C., Kruizinga, K., Barreto, L. S., Contrera, F. A. L., Imperatriz-Fonseca, V. L. (2005). Effect of group size on the aggression strategy of an extirpating stingless bee, *Trigona spinipes*. *Insectes Sociaux*, 52(2), 147–154. https://doi.org/10. 1007/s00040-004-0785-6

Nikolova, T., Yordanova, M., Petrova, V. (2019). Influence of meteorological conditions on the production of nectar and pollen of *Cucurbita pepo* var. *giromontia*. *Bulgarian Journal of Agricultural Science*, 25(2), 310-313.

Oda, F. H., Oda, T. M. (2007). Comportamento pilhador de *Trigona spinipes* Fab. Hymenoptera: apidae) em flores de *Schlumbergera truncata* (Haworth) Moran (Cactaceae). *INSULA Revista de Botânica*, 36, 95–97.

Ozga, J. A., Reinecke, D. M. (2003). Hormonal Interactions in Fruit Development. *Journal of Plant Growth Regulation*, 22, 73–81. https://doi.org/10.1007/s00344-003-0024-9

Paulino, C. S., Carneiro, L. T., Almeida, N. M., Santa-Martinez, E., Castro, C. C. (2023). Neutral influence of animal pollination in the common bean (*Phaseolus vulgaris* L., Fabaceae) production and seed germination. *Scientia Horticulturae*, 318, 112096. https://doi.org/10.1016/j.scienta.2023.112096

Paulino, C. S., Souza, C. S., Santa-Martinez, E., Almeida, N. M., Castro, C. C. (2021). Global meta-network of legume crops and floral visitors reveals abundance of exotic bees. *Journal of Applied Entomology*, *146*, 252-261. DOI: 10.1111/jen.12956

Peixoto, P. G., Martins, H. L., Pinto, B. C., Franco, A. L., Amaral, L. S., Castro, C. V. (2022). The significance of pollination for global food production and the guarantee of nutritional security: a literature review. *Environmental Sciences Proceedings*, 15(1), 1-7. https://doi.org/10.3390/environsciproc2022015007

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353. https://doi.org/10.1016/j.tree.2010.01.007

Pufal, G., Steffan-dewenter, I., Klein, A. M. (2017). Crop pollination services at the landscape scale. *Current Opinion in Insect Science*, 21, 91–97. https://doi.org/10.1016/j.cois.2017.05.021

Rech, A. R., Agostini, K., Oliveira, P. E., Machado, I. C. (2014). Biologia da polinização. Rio de Janeiro: Projeto Cultural.

Ropars, L., Affre, L., Thébault É., Geslin, B. (2022). Seasonal dynamics of competition between honey bees and wild bees in a protected Mediterranean scrubland. *Oikos,* 2022, e08915. https://doi.org/10.1111/oik.08915

Roubik, D. W. (2018). The pollination of cultivated plants: A compendium for practitioners. Food and Agriculture Organization of the United Nations.

Santos, R. S., Carneiro, L. T., Santos, P. O., J., Silva, M. M., Milfont, M. O., Castro, C. C. (2021). Bee pollination services and the enhancement of fruit yield associated with seed number in self-incompatible tangelos. *Scientia Horticulturae*, 276, 109743. https://doi.org/10.1016/j.scienta.2020.109743

Santos, R. S., Milfont, M. O., Silva, M. M., Carneiro, L. T., Castro, C. C. (2020). Butterflies provide pollination services to macadamia in northeastern Brazil. *Scientia Horticulturae,* 259, 108818. https://doi.org/10.1016/j.scienta.2019.108818

Schlering, C., Schweiggert, R., Dietrich, H., Frisch, M., Zinkernagel, J. (2020). Effects of Moderately-Reduced Water Supply and Picking Time on the Chemical Composition of Pickling Cucumber (*Cucumis sativus* L.) in Open Field Cultivation. *Agronomy*, 10(8), 1097. https://doi.org/10.3390/agronomy10081097

Serra, B. D., Campos, L. A. O. (2010) Polinização entomófla de abobrinha, *Cucurbita moschata* (Cucurbitaceae). *Neotropical Entomology*, 39(2), 153–159. https://doi.org/10.1590/S1519-566X2010000200002

Sharifkhah, M., Bakhshi, D., Pourghayoumi, M., Abdi, S., Hokmabadi, H. (2020). Effect of Pollination Time on Yield and Antioxidant Properties of Some Pistachio Cultivars. *International Journal of Horticultural Science and Technology*, 7, 51-58. https://doi.org/10.22059/ijhst.2020.285527.306

Silva, L. B, Silva, J. B., Silveira, C., Menck, M, Sales, M. F., Castro, C. C. (2021). Plant-animal interactions of understory species in an area of tropical rainforest, Northeastern Brazil. *Austral Ecology*, 46, 561-573. https://doi.org/10.1111/aec.13004

Singh, J., Singh, V., Shukla, S., Rai, A. K. (2016). Phenolic Content and Antioxidant Capacity of Selected Cucurbit Fruits Extracted with Different Solvents. *Journal of Nutrition & Food Sciences*, 6, 565. https://doi.org/10.4172/2155-9600.1000565

Valadares, E. F., Carvalho, A. T., Martins, C. F. (2023). Nest density, spatial distribution, and bionomy of *Trigona spinipes* (Apidae: Meliponini). *Journal of Apicultural Research*, 62(4), 680–691. https://doi.org/10.1080/00218839.2021.1917861

Vinícius-Silva, R., Parma, D. F., Tostes, R. B., Arruda, V. M., Werneck, M. V. (2017). Importance of bees in pollination of *Solanum lycopersicum* L. (Solanaceae) in open-field of the Southeast of Minas Gerais State, Brazil. *Hoehnea*, 44, 3, 349-360. http://dx.doi.org/10.1590/2236-8906-07/2017

Wolowski, M., Agostini, K., Rech A. R., Varassin I. G., Maués M., Freitas L., Saraiva A. M. (2019). Relatório temático sobre polinização, polinizadores e produção de alimentos no Brasil. Espírito Santo: REBIPP. http://doi.org/10.4322/978-85-60064-83-0

FIGURE LEGENDS

Figure 1. Frequency of floral visits in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil. A, B, C: Sites I, II and II, respectively.

Figure 2. Effect of floral display on total frequency of floral visits (A), frequency of *Apis mellifera* (B) and *Trigona spinipes* (C) in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil.

Figure 3. Effect of the number of visits by all floral visitors (A), by *Trigona spinipes* (B) and by *Apis mellifera* (C) on production quality in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil.

Figure 4. Antioxidant activity of pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) determined by $ABTS^{+}$ assays in fruits resulted from natural (NP) and cross (CP) pollination experiments.

Figure 5. Relationship between number of seeds and fruit weight in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil.

Table 1. Floral visitors observed in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations of a semiarid region of NE Brazil.

	Area I	Area II	Area III
Algochlora sp.		x	
Apis mellifera	x	x	x
Melipona scutelaris		x	
Paratrigona cf. *incerta*		x	
Polybia sp.	x	x	
Trigona spinipes	x	x	x
Xylocopa (*Neoxylocopa*) sp.	x	x	

Table 2. Influence of the total number of visits, visits by *A. mellifera*, visits by *T. spinipes* and the joint effect of visitation by both species on pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) production parameters in three plantations of a semiarid region of NE Brazil. χ^2, chi-square values results from analysis of variance. *Significant effect on a given response variable ($p < 0.05$).

Variable	All pollinators		*A. mellifera*		*T. spinipes*		*A. mellifera* × *T. spinipes*	
	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Fruit set	3.21	0.073	1.67	0.196	2.47	0.116	0.002	0.957
Seed number	1.27	0.260	1.71	0.190	0.56	0.454	0.47	0.492
Fruit weight (Log)	17.79	< 0.001*	1.38	0.239	18.60	< 0.001*	0.24	0.623
Pericarp length (Log)	0.04	0.839	5.98	0.014*	0.23	0.628	0.0005	0.983
pH	0.11	0.738	0.08	0.774	0.59	0.442	2.00	0.158

Table 3. Effect of pollination treatments (natural and cross pollinations) and seed number on yield quantity and quality parameters in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations of a semiarid region of NE Brazil. Fruit set per treatment is given as a percentage. Mean values (± SD) per treatment are presented for the other response variables. χ^2, chi-square values, analysis of variance results. *Significant effect on a given response variable ($p < 0.05$).

Variable	Fixed effects							
	Treatment						Seed number	
	Natural pollination	*n*	Cross pollination	*n*	χ^2	*p*	χ^2	*p*
Fruit set (%)	85.3	150	75.9	137	5.84	0.016*	-	-
Seed number	296.8 ± 123.3	128	319.6 ± 121.3	104	1.54	0.215	-	-
Fruit weight (kg)	2.5 ± 0.9	128	2.6 ± 1.1	104	0.11	0.738	13.32	< 0.001*
Pericarp length (mm)	40.0 ± 11.0	128	41.3 ± 13.8	104	0.0001	0.991	1.80	0.180
pH	6.2 ± 0.2	128	6.2 ± 0.2	104	0.99	0.321	2.06	0.151
πmol Eq. a Trolox/L ext (mean)	1003.2 ± 253.6	20	563.2 ± 235.8	20*	19.39	< 0.001*	-	-

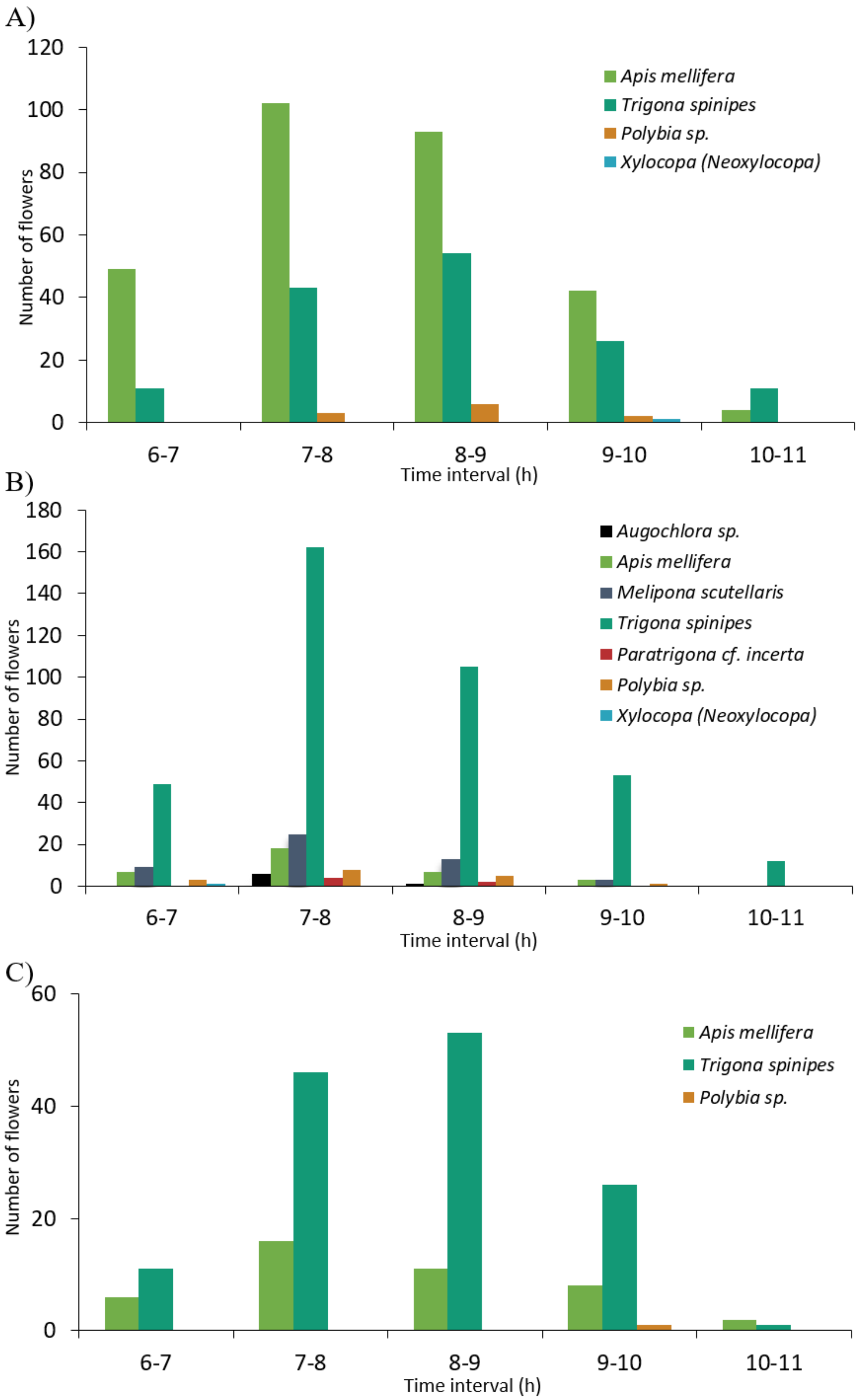

Figure 1. Frequency of floral visits in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil. A, B, C: Sites I, II and II, respectively.

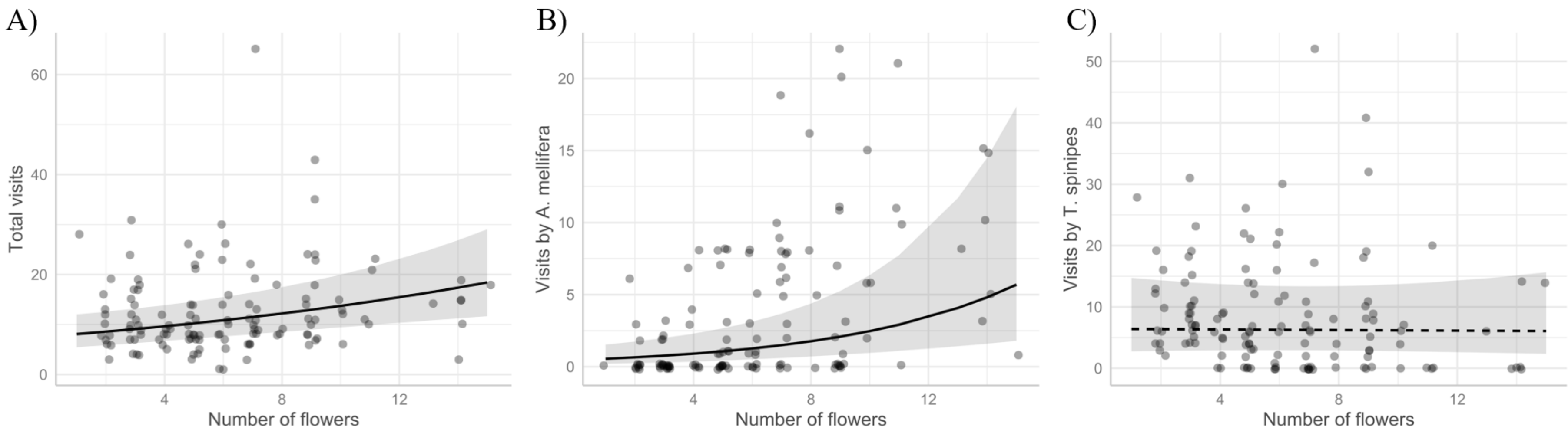

Figure 2. Effect of floral display on total frequency of floral visits (A), frequency of *Apis mellifera* (B) and *Trigona spinipes* (C) in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil.

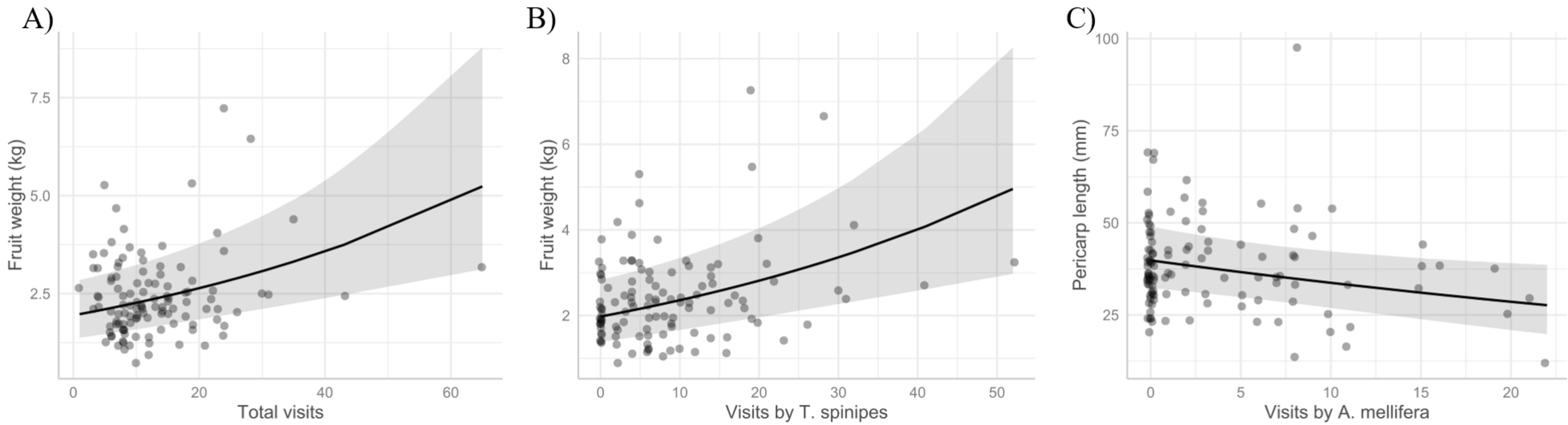

Figure 3. Effect of the number of visits by all floral visitors (A), by *Trigona spinipes* (B) and by *Apis mellifera* (C) on production quality in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil.

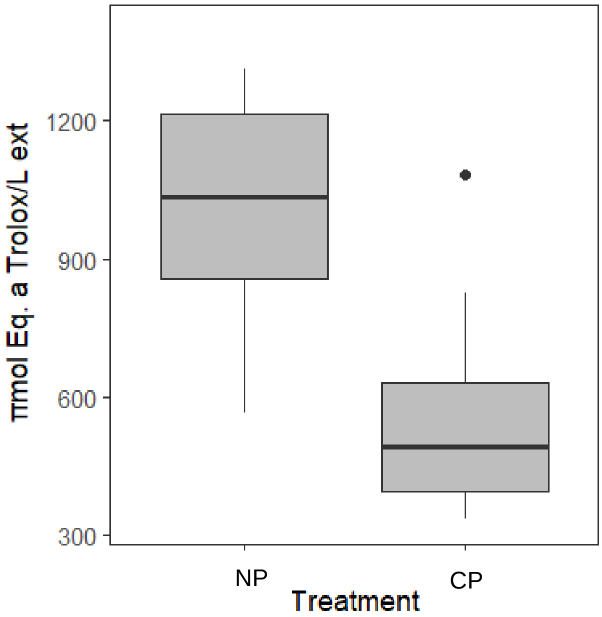

Figure 4. Antioxidant activity of pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) determined by ABTS$^+$ assays in fruits resulted from natural (NP) and cross (CP) pollination experiments.

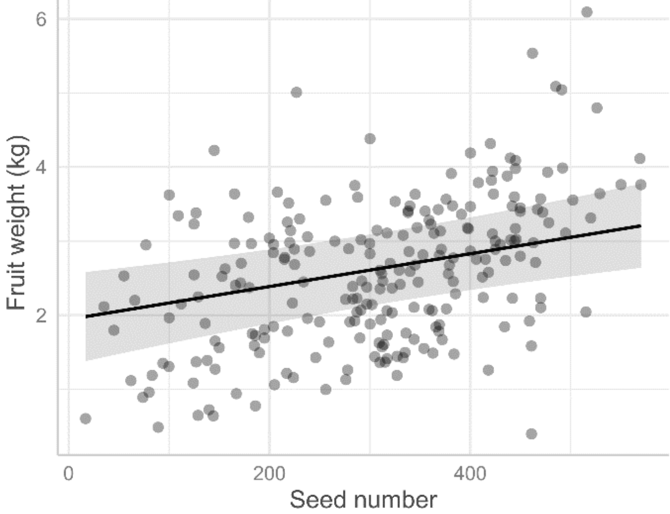

Figure 5. Relationship between number of seeds and fruit weight in three pumpkin (*Cucurbita moschata* Duchesne, Cucurbitaceae) plantations in a semiarid region of northeastern Brazil.