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Refuge as a Tool for Pollinator Conservation: Enhancing Biodiversity in RGL (*Rimau Gerga Lebong*) Citrus Orchards

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Abstract

The RGL (Rimau Gerga Lebong) citrus orchard in Malang Regency, East Java (7°55'12“ S; 112°33'45” E) was the location for this study, which focused on the problem of declining pollinator populations in fruit cultivation areas. The study aimed to analyze pollinator diversity in three types of flowering plants sunflowers (*Helianthus annuus*), marigolds (*Tagetes erecta*), and wild cosmos (*Cosmos caudatus*) grown around citrus orchards, while evaluating their contribution to agroecosystem stability within the framework of Integrated Pest Management (IPM). Field observations were conducted from June to December 2024 using a combination of visual censuses and sweep net methods along 100 m × 2 m transects. From the observation results, 29 pollinator species from 10 families were identified, with Apidae as the dominant group that was consistently present in all treatments. These findings indicate that planting flowering plants around RGL orange orchards could support the recovery of pollinator communities while strengthening ecosystem services in integrated agricultural systems. Refuge planting increased species richness (R = 3.87 vs. 3.40 in controls) and total pollinator abundance, although the Shannon-Wiener Index (H’) in refuge areas (1.696) was lower than in controls (2.075), attributable to the disproportionate dominance of *Amata huebneri*. Sunflower refuge supported the highest pollinator diversity and abundance, marigold effectively attracted Syrphidae and Meliponini stingless bees, while wild cosmos enriched Lepidoptera and solitary bee composition. Bray-Curtis cluster analysis and PCA ordination confirmed that each refuge type produced distinct qualitative shifts in pollinator community structure. This study recommends implementing multispecies refuge systems combining all three plant species proportionally to prevent single-species dominance, broaden pollinator guild diversity, and optimize integrated pollination and biological control services in RGL citrus agroecosystems.

Keywords: Apidae; citrus agroecosystem; integrated pest management; pollinator diversity; refuge.

INTRODUCTION

RGL citrus (Rimau Gerga Lebong) are among the leading horticultural commodities of high economic value, currently widely cultivated in Malang Regency. The success of RGL citrus production is highly dependent on the effectiveness of pollination, especially that mediated by pollinating insects. A number of studies show that insect involvement in the pollination process yields much better results the fruit grows larger, tastes better, and the seeds are more viable than if the plants rely solely on the wind or their own pollen (Garibaldi et al., 2013; Pioltelli et al., 2024). In the context of RGL oranges, this means that a rich and diverse pollinator community is not just an ecological bonus but a prerequisite for productivity that can be maintained from season to season.

However, in the last two decades, declines in pollinator populations and diversity have been widely reported in agricultural ecosystems around the world. Key factors driving this phenomenon include agricultural intensification, habitat loss and fragmentation, and excessive pesticide use (Goulson et al., 2015; Xerces Society for Invertebrate Conservation, 2021). This condition not only reduces pollinator visit frequency to cultivated plants but also has the potential to create ecological imbalances that increase pest pressure. Globally, the phenomenon of "pollinator decline" has been recognized as a critical issue that directly impacts food security and the sustainability of agricultural production systems (Nath et al., 2023).

One ecological approach to address this problem is habitat engineering through the planting of refuge. Refuge is defined as strips or patches of flowering plants planted within or around cultivated land, providing alternative food sources in the form of nectar and pollen, and microhabitats for pollinators and natural enemies of pests (Windriyanti et al., 2023). Research by Lowe et al., 2021 shows that planting refuge on the edges of agricultural land significantly increases pollinator visits and crop yields. Furthermore, refuge have been shown to support agroecosystem stability by naturally suppressing pest populations, thereby reducing farmers' dependence on synthetic chemical pesticides (Chellam et al., 2024).

Although the benefits of refuge for pollinator conservation have been widely reported globally, research on tropical citrus systems, particularly the RGL variety in Indonesia, remains very limited. Most previous studies have focused on food crops such as rice and chili peppers (Aminah et al., 2021; Aminatun et al., 2023). Several reports on citrus agroecosystems indicate that habitat heterogeneity influences the composition of flower-visiting insects, including pollinators (Alignier et al., 2023). The study confirmed that the complexity of tropical agricultural landscapes positively correlates with pollinator diversity and pollination efficiency in fruit crops. However, empirical data on the responses of citrus pollinators to refuge in horticultural agroecosystems have not been adequately documented. However, empirical data on the responses of citrus pollinators to refuge in horticultural agroecosystems have not been adequately documented. The agroecological conditions of citrus, including flowering phenology, susceptibility to fruit flies, and interactions between pollinators and natural enemies, differ from those of seasonal food crop systems, thus requiring separate studies.

Based on this knowledge gap, this study aims to analyze pollinator diversity in various types of refuge planted in the RGL citrus orchard in Malang Regency. Specifically, this study focuses on identifying pollinator species, analyzing differences in diversity among refuge types, and their contribution to the stability of the citrus agroecosystem. The findings from this study are expected to serve as a concrete scientific basis not merely general recommendations for designing refuge-based conservation strategies that truly work in the field. Furthermore, the results are expected to support productive and sustainable RGL citrus management, particularly within the framework of Integrated Pest Management (IPM).

MATERIALS AND METHODS

This study was conducted at the RGL (Rimau Gerga Lebong) citrus orchard in Malang Regency, East Java (7°55'12" S; 112°33'45" E), as shown in Figure 1. The location was not chosen arbitrarily—the orchard was selected because it met three criteria: representative land area, high production intensity, and reports of fruit fly infestation from local farmers that were quite alarming. Observations were conducted during two flowering and fruiting seasons, from June to December 2024. This time frame was deliberately chosen so that pollinator population dynamics could be observed at various phases of the RGL orange phenology not only during peak flowering, but also when the plants were entering the fruit formation and ripening phases.

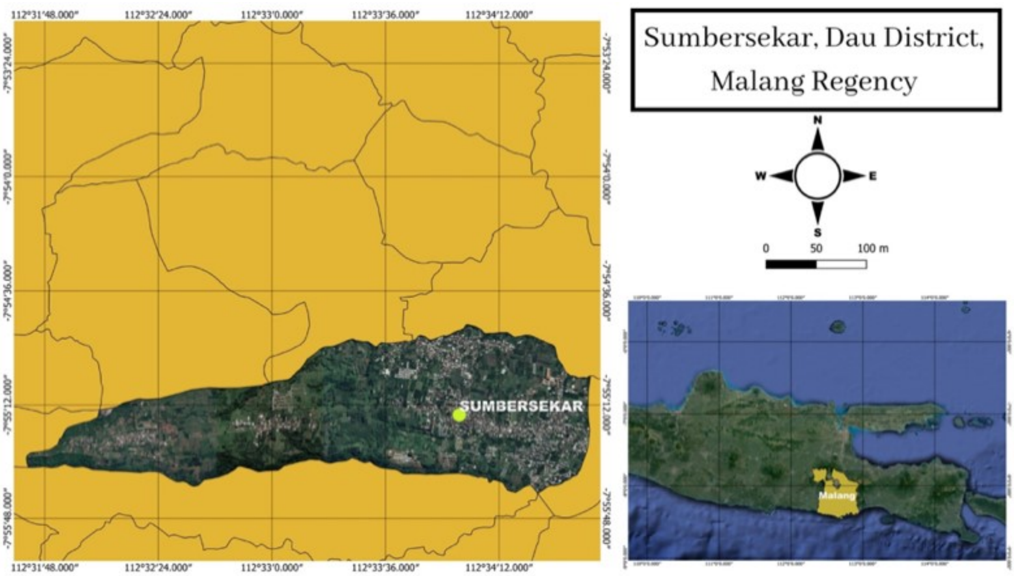

Figure 1. Map of Research Location in Sumbersekar Village, Dau District, Malang Regency, East Java.

Research Design and Sampling

To systematically document pollinator communities, this study employed an ecological survey method using a quadrat transect. In each garden, a 100 m × 2 m transect was installed and then divided into four observation quadrants, facilitating structured recording without missing any part of the observed area. The number of transects was adjusted to the area of each orchards, with a minimum of three replications to ensure the data were statistically robust. Observations were made directly on RGL citrus flowers that were in bloom in each quadrant. This approach was chosen not only for its technical ease but also because it has been shown to capture spatial and temporal variations in pollinator communities more accurately, a challenge that is difficult to overcome when observations are made randomly, without a clear structure (Singh et al., 2025).

Pollinators and Flying Fruit Observations

Pollinator observations were conducted using a combination of two complementary methods. First, visual census—observers directly recorded insects that landed on and visited orange blossoms in each quadrant, for 15 minutes each. Observation sessions were scheduled twice a day: in the morning, between 7:00 a.m. and 11:00 a.m., and in the afternoon, between 2:00 p.m. and 5:00 p.m. The choice of time window was not accidental; it is during this period that pollinating insect activity in tropical agroecosystems tends to be highest (Hagen & Kraemer, 2010). However, visual censuses have limitations—not all insects can be identified by sight alone. This is where the sweep net method comes into play. The specimens caught are placed in collection bottles containing 70% ethanol, then taken to the laboratory for more detailed identification using identification keys and relevant reference literature (Borror, 1992; Michener, 2007). The collected data included the number of individuals, the types of pollinator species, and the frequency of visits to flowering RGL citrus trees.

Parameters

The main parameters in this study include pollinator diversity and relative abundance, which represent the ecological dynamics of the pollinator community in the RGL citrus orchard. Pollinator diversity was analyzed using the Shannon-Wiener Index (H'), which was calculated using the following formula:

$$H' = -\Sigma (p_i \ln p_i)$$

where p_i is the proportion of individuals of species i relative to the total number of individuals of all species found. This index was chosen because it is sensitive to changes in species composition and has been widely used in studies of insect diversity in agroecosystems (Magurran AE, 2004). The relative abundance of pollinators was calculated as the ratio of the number of individuals of each species to the total number of pollinators observed, thereby identifying the dominant species in the pollinator community at each observation site.

Data Analysis

The observation data, including diversity index values and average insect abundance, were analyzed using one-way analysis of variance (ANOVA) to test the differences between treatments in the three types of refuge tested, namely *Helianthus annuus (sunflower), Tagetes erecta (marigold), Cosmos caudatus (wild marigold)*. If anova shows significant differences, the analysis continues with the Tukey HSD (Honestly Significant Difference) test at the 95% confidence level to determine which treatment groups differ significantly. All statistical analyses were performed using SPSS version 25. The results of the analysis are presented in bar charts with error bars (standard errors), and significant differences between treatments are indicated by different letters on each graph (Callaway et al., 2024).

RESULTS AND DISCUSSION

Taxonomic Composition and Pollinator Community Structure

Observations in the RGL (Rimau Gerga Lebong) citrus orchard in Malang Regency during the period June–December 2024 identified 29 pollinator species from 10 families distributed across all treatments (Table 1). This taxonomic richness includes three major orders of insects known as pollinators: Hymenoptera (Apidae, Halictidae, Megachilidae, Eumenidae), Diptera (Syrphidae, Stratiomyidae, Cantharidae), and Lepidoptera (Pieridae, Erebidae, Nymphalidae, Hesperiidae). The presence of all these species indicates that the RGL citrus orchard agroecosystem can provide sufficient habitat for various pollinator groups with diverse ecological functions, so that if one pollinator species declines, other species can still take over its role in maintaining pollination services (Ulyshen et al., 2023).

Table 1. Comparative Abundance of Pollinators in Refuge and Control Plots.

No	Species	Common name	Family	Treatment (with refuges)			Control
				Sun-flower	Marigold	Cosmos wild	
1	*Allograpta exotica* (Wiedemann, 1830)	Syrphid flies	Syrphidae	4	1	1	-
2	*Amata huebneri* (Boisduval, 1829)	Hübner's wasp moth	Erebidae	325	89	39	58
3	*Apis cerana* (Fabricius, 1793)	Asian honeybee	Apidae	0	2	2	-
4	*Apis mellifera* (Linnaeus, 1758)	Western honey bee	Apidae	28	4	2	14
5	*Apis nigrocincta* (Smith, 1860)	Sulawesian honey bee	Apidae	0	3	2	9
6	*Apis sp. (Linnaeus, 1758)*	Honey bee	Apidae	15	14	8	9
7	*Appias nero* (Wallace, 1867)	Citrus albatross	Pieridae	3	0	1	2
8	*Bombus terrestris* (Linnaeus, 1758)	Buff-tailed bumblebee	Apidae	1	1	1	1
9	*Ceratina sp.* (Latreille, 1802)	Small carpenter bee	Apidae	2	0	0	-

No	Species	Common name	Family	Treatment (with refuges)			Control
				Sun-flower	Marigold	Cosmos wild	
10	*Chauliognathus pensylvanicus* (Riley, 1872)	Goldenrod soldier beetle	Cantharidae	7	0	0	-
11	*Chloromyia formosa* (Scopoli, 1763)	Broad centurion	Stratiomyidae	6	2	1	1
12	*Copestylum vesicularium* (Curran, 1947)	Iridescent bromeliad fly	Syrphidae	0	2	0	6
13	*Eurema adamsi* (Lathy, 1898)	Grass yellow butterflies	Pieridae	0	1	0	-
14	*Eurema* hecabe (Linnaeus, 1758)	Common Grass Yellow	Pieridae	0	0	1	4
15	*Eurema mexicana* (Boisduval, 1836)	Mexican yellow	Pieridae	4	2	0	4
16	*Frieseomelitta nigra* (Cockerell, 1912)	Stingless bee	Apidae	68	72	11	95
17	*Halictus ligatus* (Say, 1837)	Ligated furrow bee	Halictidae	9	0	0	-
18	*Halictus tripartitus* (Cockerell, 1895)	Tripartite sweat bee	Halictidae	0	0	2	4
19	*Heriades truncorum* (Linnaeus, 1758)	Ridge-saddled carpenter bee	Megachilidae	0	4	0	-
20	*Lethe confuse* (Aurivillius, 1898)	Banded Treebrown	Nymphalidae	-	-	-	1
21	*Pelopidas mathias* (Fabricius, 1798).	Small branded swift	Hesperiidae	2	1	6	-
22	*Pieris ergane* (Greyer, 1828)	Mountain small white	Pieridae	1	0	0	1
23	*Pieris angelica* (Eitschberger, 1983)	Arctic white	Pieridae	1	9	2	5
24	*Pieris brassicae* (Linnaeus, 1758)	Large White Butterfly	Pieridae	1	2	0	-
25	*Rhynchium haemorrhoidale* (Fabricius, 1775)	Potter wasp	Eumenidae	2	0	1	1
26	*Sphaerophoria scripta* (Linnaeus, 1758)	Long hoverfly	Syrphidae	0	9	5	6
27	*Trigona fulviventris* (Guérin-Méneville, 1845)	Stingless bee	Apidae	11	17	9	12
28	*Vanessa atalanta* (Linnaeus, 1758)	Red admiral	Nymphalidae	0	1	1	1
29	*Xylocopa mexicanorum* (Cockerell, 1912)	Mexican Carpenter Bee	Apidae	-	-	-	30

The Apidae family consistently dominated the pollinator community across all treatments, with key species including *Frieseomelitta nigra*, *Apis mellifera*, *Apis nigrocincta*, *Apis cerana*, *Trigona fulviventris*, and *Xylocopa mexicanorum*. The dominance of Apidae in tropical agroecosystems is not particularly surprising this pattern has been repeatedly documented in various studies. There are several reasons why this group is so resilient: its colonies are highly adaptive, its eusocial foraging strategy makes it efficient, and its tolerance to habitat disturbance is relatively high compared to other pollinator groups (Jordão et al., 2024; Serralta-Batun, 2024; Sladonja et al., 2023). For citrus in particular, the advantages of social bees such as *Apis* spp. are even more pronounced their visitation frequency is high, and in a single flight session they are able to consistently transfer pollen from one flower to another, a combination that is difficult for other pollinators to match (Vanlalhmangaiha et al., 2023).

Outside of Apidae, the Diptera group especially from the Syrphidae family also deserves attention. Three recorded species, namely *Allograpta exotica, Sphaerophoria* scripta, and *Copestylum vesicularium*, have a fairly high visit frequency, especially in marigold refugia. What is interesting about Syrphidae is their dual role: as adults, they actively visit flowers and help transfer pollen; while their larvae are voracious predators of Aphididae indirectly helping to suppress aphid populations without the need for chemical intervention (Burgio et al., 2025; Jiang et al., 2023). The presence of Lepidoptera, especially *Amata huebneri* (Erebidae), which is the most abundant species overall, adds to the functional diversity of the pollinator community through variations in body size, visitation patterns, and pollen transfer mechanisms (Kumar et al., 2024).

Pollinator Diversity: Ecological Index Analysis

Shannon-Wiener Index Patterns and Ecological Trade-offs

One of the most interesting findings was the result of the analysis of the pollinator community diversity index. The Shannon-Wiener Index (H') value in the sanctuary area was lower (H' = 1.696) than in the control area without a sanctuary (H' = 2.075), although both were still classified as having moderate diversity based on (Magurran, 2004) criteria (Table 2). This finding cannot be ignored it challenges the assumption held by many researchers that the presence of a sanctuary will automatically increase pollinator diversity. There are clearly other mechanisms at work behind this pattern, and these need to be explored further.

Table 2. Pollinator diversity index in refuge and control treatments in RGL citrus orchard.

Index	Treatment (with refuge)	Control
Shannon-Wiener (H')	1.696 (Moderate)	2.075 (Moderate)
Dominansi (D)	0.345 (Low)	0.2001 (Low)
Evenness (E)	0.5147 (Moderate)	0.6929 (Low-High)
Richness	3.87 (Moderate)	3.40 (Moderate)

The decline in H' in refuge areas is a direct consequence of the abundant and concentrated availability of nectar and pollen from refuge flowers, which tend to attract generalist pollinator species that are highly responsive to abundant food sources, especially *Amata huebneri*, in much greater numbers than other species. The imbalance in the number of individuals between species ultimately has a direct impact on the distribution of the community as a whole—evenness decreases significantly, from 0.693 in the control to only 0.515 in the refuge area. This pattern is not an anomaly; (Noss et al., 2021) reported similar findings, where flowering plants planted as refuges sometimes triggered imbalances in pollinator communities. The root cause is the same: once food sources become abundant, the most adaptive and aggressive species tend to monopolize these resources, while other species are pushed to the margins. A similar study conducted by (Rahmawati et al., 2025) found the same results in refuge planting systems in Indonesian tropical agrosystems, namely that pollinator community structure experienced imbalances due to the dominance of certain species that were overly responsive to the availability of existing resources.

On the other hand, species richness in the refuge area was actually higher (R = 3.87 vs. 3.40 in the control)—and this is not a figure to be taken lightly. The more pollinator species present in an ecosystem, the greater its resilience to external pressures. Recent studies reinforce this point: species-rich pollinator communities have been shown to be more resilient in the face of shocks from climate change and increasingly alarming habitat degradation (Hederström et al., 2024; Loy & Brosi, 2022). Thus, there is an ecological trade-off that needs to be understood in proportion: on the one hand, refuge increase the number of species and the total abundance of pollinators, but on the other hand, community diversity temporarily decreases because some species are present in much greater numbers than others. In horticultural agricultural systems, the combination of efficient social pollinator abundance and adequate species richness is more decisive for successful plant pollination than community uniformity alone (Cohen et al., 2021).

Differences in Diversity Among Refuge Types

The one-way ANOVA test showed significant differences in pollinator diversity among the three refuge types ($p = 0.0252$; F-count > F-table at $\alpha = 0.05$). The Tukey HSD post hoc test confirmed that sunflower (*Helianthus annuus*) refuge had the highest pollinator diversity, differing significantly (Figure 2) from wild cosmos (*Cosmos caudatus*), while marigold (*Tagetes erecta*) occupied an intermediate position that did not differ significantly from either of the other two (Figure 2).

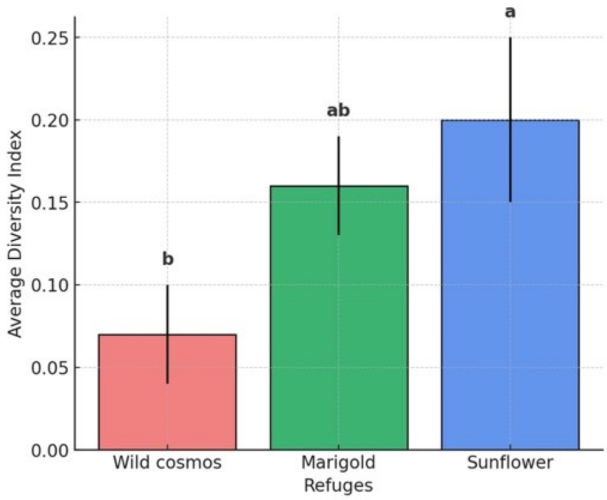

Figure 2. Diversity Index of Pollinators under Different Refuge Plants

The attraction of sunflowers to various pollinator species is apparently not a coincidence there are several factors that work together and reinforce each other. The most striking is their appearance: large-diameter capitula with bright yellow colors create a visual signal that is difficult to ignore, especially for the Hymenoptera group, whose eyes are very sensitive to the UV–green–yellow spectrum (Guignard et al., 2021). Second, the abundant production of nectar and pollen in *H. annuus*, which is readily accessible to various insect mouthparts, makes it a competitive resource for both generalist and specialist pollinators (Shpak et al., 2023). Third, the long flowering phenology ensures a continuous food source even outside the peak citrus flowering season, a condition that is highly valuable for social bee colonies that require a continuous supply of resources (Puttha et al., 2023).

Marigold (*Tagetes erecta*) shows performance equivalent to sunflowers in terms of pollinator abundance despite having a different species composition. The main mechanism of marigold attraction is the production of specific aromatic volatile compounds mainly terpenoids and monoterpenes which function as chemical signals that attract certain groups of insects, especially Syrphidae and stingless bees Meliponini (Hou et al., 2023). This explains the high abundance of *Sphaerophoria scripta* and *Frieseomelitta nigra* in marigold refuge compared to other refuge. Research in Indonesian agroecosystems confirms that marigolds and zinnias as refuge significantly increase the abundance of beneficial arthropods, including pollinators, in chili and rice crops (Hasriyanty et al., 2023; Rahmawati et al., 2026).

Wild cosmos (*Cosmos caudatus*) showed the lowest attractiveness to pollinators, most likely due to its relatively small flower size, more limited nectar availability, and less striking visual appeal compared to the other two refuge. According to (Sponsler et al., 2023), flowering plants with limited food resources can support only pollinators with specific preferences, thereby limiting diversity. Nevertheless, wild cosmos remains relevant as a refuge flower due to its ability to attract several small Lepidoptera species and solitary bees that are not attracted to large flowers, thereby contributing to the total species richness of the pollinator community.

Pollinator Abundance and Preferences for Refuge Types

The results of the ANOVA test showed significant differences in pollinator abundance between refuge types ($p = 0.00775$), with sunflowers and marigolds supporting the highest abundance not significantly different from each other while wild cosmos produced the lowest abundance (Figure 3). These differences are mechanistically explained by the morphological characteristics of the flowers and the quality of the food sources of each refuge (Kuppler & Kotowska, 2021). *Amata huebneri* (Erebidae) was recorded as the most abundant species across all refuge treatments with a total of 453 individuals, reflecting highly active and flexible foraging behavior as a generalist pollinator that rapidly exploits large quantities of available nectar sources. Although its dominance increased total flower visits, the community's dependence on a single species actually increased the vulnerability of pollination services in the long term a condition that underscores the importance of functional redundancy among pollinator species (Jordan et al., 2021).

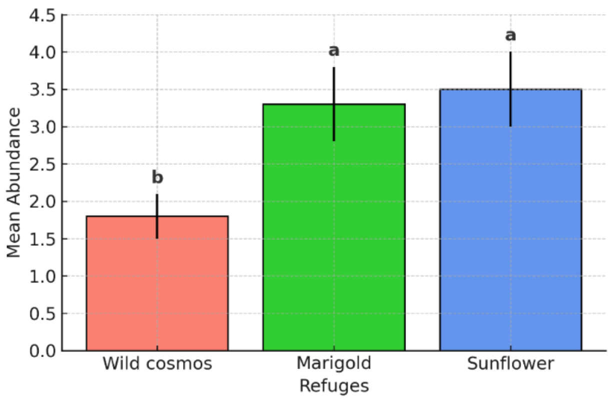

Figure 3. Mean Abundance of Pollinators under Different Refuge Plants.

Conversely, *Frieseomelitta nigra*, as the main natural pollinator, was found to be more abundant in the control area (95 individuals) than in the refuge area, indicating that this species has a strong tendency to continue to utilize citrus flowers as its main food source and does not easily switch even when alternative food sources are available from the refuge (Bednaršek et al., 2024). *Xylocopa mexicanorum*, which was only found in the control area, indicates that the types of refuge used in this study were not able to meet the ecological needs of this species, so in the future, it is necessary to consider adding types of refuge with tube-shaped flowers that are more suited to the preferences of this species. The Syrphidae group *Allograpta exotica*, *Sphaerophoria scripta*, and *Copestylum vesicularium* show dual ecological value: in addition to acting as secondary pollinators, their larvae are active predators of Aphididae, so that refuge also function as habitats for maintaining natural enemies of pests, strengthening the overall trophic network of the RGL citrus agroecosystem (Boon et al., 2023; Setyadin et al., 2025).

Importance Value Index (IVI): The Ecological Role of Dominant Species

Importance Value Index (IVI) analysis integrates abundance, frequency, and relative dominance to provide a more representative picture of a species' ecological role than abundance data alone. *Amata huebneri* dominated the IVI values across all refuge treatments sunflowers (137.66%), wild cosmos (88%), and marigolds (81%). IVI values exceeding 100% in sunflowers are a serious signal: the ecological space for other species is practically shrinking, a pattern consistent with the report by (Puttha et al., 2023), which states that refugia with high attractiveness are prone to causing imbalanced community structures.Meanwhile, *Frieseomelitta nigra* dominated the control area (IVI = 76.96%) and remained in second place in the marigold treatment (66.60%) indicating its consistent role as an important species in the natural pollinator community in the RGL orange grove. As for *Xylocopa mexicanorum*, although it was quite prominent in the control area (IVI = 27.72%), it was not found at all in the three refuge areas. Its absence reinforces the assumption that the types of refuge plants used in this study have not been able to meet the ecological needs of this species, so it is necessary to consider adding plants with flower morphology that is more suited to its preferences. Species with moderate IVI, such as *Apis* sp., *Trigona fulviventris*, and *Sphaerophoria scripta*, which are evenly distributed across treatments, act as supporting pollinators that maintain the stability and functional redundancy of pollination services; this combination of dominant and supporting pollinators reflects the principle of complementarity in pollinator community ecology, where synergies between species result in more effective pollination services that are resilient to disturbances (Aguiar et al., 2024; Asmini et al., 2022).

Tabel 1. IVI of Pollinator Insects in Refuge and Control Plots

No	Species	Sunflower	Marigold	Wild Cosmos	Control
1	*Amata huebneri*	137.66%	81.00%	88.00%	48.94%
2	*Frieseomelitta nigra*	32.76%	66.60%	29.04%	76.96%
3	*Apis mellifera*	16.42%	8.95%	10.09%	15.60%
4	*Apis* sp.	11.12%	17.42%	22.72%	11.82%
5	*Trigona fulvisentris*	9.48%	19.96%	24.82%	14.10%
6	*Pieris angelica*	5.41%	13.18%	10.09%	8.79%
7	*Sphaerophoria scripta*	0.00%	13.18%	16.40%	9.55%
8	*Xylocopa mexicanorum*	0.00%	0.00%	0.00%	27.72%
9	*Apis nigrocincta*	0.00%	8.09%	10.09%	11.82%
10	*Allograpta exotica*	6.63%	6.40%	7.99%	0.00%

Bray-Curtis Similarity Index

Bray-Curtis cluster analysis produced an ecologically meaningful three-cluster grouping pattern: marigolds and controls formed the cluster with the highest similarity (≈0.90), indicating that marigold refuge function as amplifiers of existing generalist pollinator communities rather than as formers of new communities due to their flower morphology being compatible with the pollinator guilds that already dominate the control area (Torezan-Silingardi et al., 2021) (Figure 4). In contrast, the pollinator community in the sunflower area showed the lowest similarity among all treatments (≈0.38–0.45), consistent with the abundance of *Amata huebneri,* which completely altered the pollinator community composition. Meanwhile, wild cosmos was in the middle, sharing similarities with the other two groups but with a different Lepidoptera species composition (Traut et al., 2023).

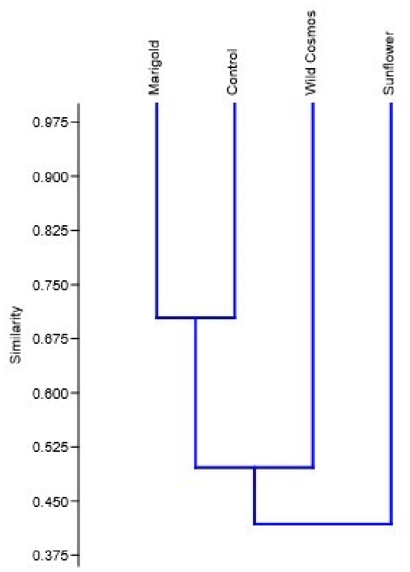

Figure 4. Bray–Curtis Similarity of Pollinator Communities under Refuge Treatments.

This pattern confirms that the selection of refuge types not only affects the number of pollinators that arrive, but also produces different qualitative changes in the composition of the pollinator community, so the selection of refuge types needs to be adjusted to the ecological objectives to be achieved. If the goal is to maintain the stability of existing pollinator communities, marigolds are the better choice; if the goal is to significantly increase pollinator abundance, sunflowers are more suitable; while wild cosmos are more appropriate if the goal is to gradually enrich species composition diversity as confirmed by (Bottero et al., 2023), who documented noticeable variations in pollinator community composition in response to differences in refuge types.

PCA ordination confirmed and reinforced the patterns found in the Bray-Curtis analysis (Figure 5). Based on the ordination graph, sunflowers are separated far to the right along the PC1 axis (component 1 value ≈ +175) from the other three treatments, which are all clustered on the left side (component 1 values range from −95 to −40), indicating that the composition of the pollinator community in the sunflower area is fundamentally different from all other treatments. This extreme separation is directly caused by the abundance of *A. huebneri*, which dominates the sunflower area, making the differences in species composition between treatment locations very striking (Fried et al., 2022).

Meanwhile, the control and marigold clusters are relatively close despite differing on the PC2 axis—the control is higher (≈ +41), and the marigold is in the middle (≈ +9)—confirming that the two have more similar community compositions than the sunflower. Wild cosmos occupied the lowest position on the PC2 axis (≈ −49) but remained close to the control and marigold on the PC1 axis, reflecting a different composition, particularly in the proportion of Lepidoptera species, but not to the extent of the differences shown by sunflowers. The managerial implications of this ordinal pattern are clear: relying on a single type of highly attractive refuge, such as sunflowers, risks creating a pollinator community that is vulnerable to the dominance of a single species. Therefore, diversifying refuges by combining sunflowers, marigolds, and wild cosmos is advisable to produce a more stable distribution of pollinator visits that is resistant to long-term ecological disturbances (Shpak et al., 2023).

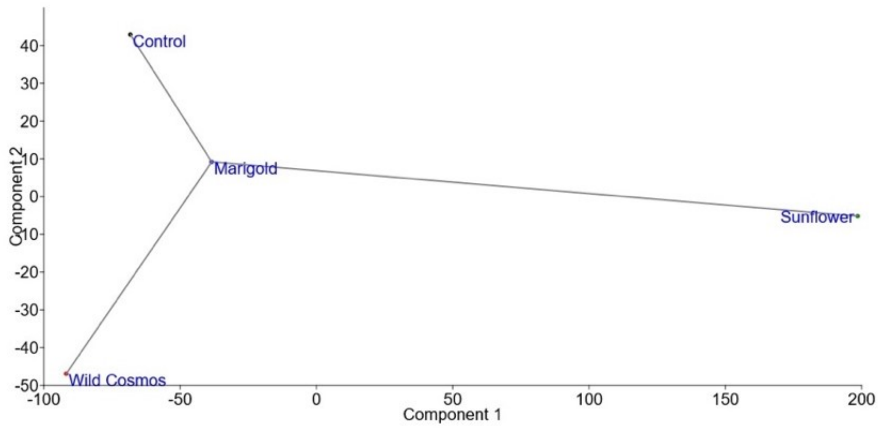

Figure 5. Multivariate Analysis of Pollinator Distribution Using PCA.

Ecological Implications and Refuge Management in the IPM Framework

Integrally, this study proves that refuge provide layered ecological impacts in RGL citrus orchards: at the pollination service level, increased abundance of *Apis* spp., *F. nigra*, and *T. fulviventris* directly improves fruit set, fruit size uniformity, and organoleptic quality of the harvest (David et al., 2022; Maia-Silva et al., 2024; Villagómez et al., 2024) at the biological control level, refuge attract parasitoids and natural enemies of *Bactrocera* spp. (Rahmawati et al., 2024) and Aphididae including through the observed presence of Syrphidae larvae thus integrating pollination and pest suppression functions into a single strategy that represents the ideal application of IPM principles without relying on chemical pesticides (Ikhsan, 2024; Lundin et al., 2021). However, there are important caveats that should not be overlooked. The fact that H' and evenness values were higher in the control area reminds us that high diversity does not necessarily equate to better pollination intensity. For citrus agroecosystems like the RGL, the ideal condition is not simply an even community, but rather a combination of sufficient species richness and a truly high abundance of key pollinators.

From this, three operational recommendations deserve serious consideration. First, implement multispecies refugia by proportionally combining sunflowers, marigolds, and wild cosmos rather than just one species to suppress the dominance of a single species and broaden the spectrum of pollinator guilds available (Josephrajkumar et al., 2022). Second, consider adding tubular or zygomorphic flowering plants to reach *Xylocopa mexicanorum*, which has been shown to be unattractive to the three existing refugia types. Third, design refugia with overlapping flowering times between plants, so that food resources are available without interruption throughout the RGL citrus flowering season. If these three principles are implemented together, the resulting pollinator community architecture will be much more functional and long-lasting in line with the spirit of sustainable agriculture and the integrated IPM framework (Phan et al., 2025).

CONCLUSIONS

This study identified 29 pollinator species from 10 families in the RGL citrus orchard in Malang Regency, with Apidae as the dominant group in all treatments. Refuge planting increased species richness (R = 3.87 vs. 3.40 in the control) and total pollinator abundance, although the H' value in the refuge area (1.696) was lower than in the control (2.075) due to the dominance of *Amata huebneri*, which suppressed community evenness. Among the three types of refuge, sunflowers (*Helianthus annuus*) were most effective at attracting pollinator diversity; marigolds (*Tagetes erecta*) excelled at attracting Syrphidae and Meliponini; and wild marigolds (*Cosmos caudatus*) enriched the composition of Lepidoptera and solitary bees. This study recommends the proportional application of multispecies refuge to prevent single-species dominance and optimize pollination services within the framework of sustainable Integrated Pest Management (IPM) in the RGL citrus agroecosystem.

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REFERENCES

Aguiar, L. M. S., Diniz, U. M., Bueno-Rocha, I. D., Filomeno, L. R. A., Aguiar-Machado, L. S., Gomes, P. A., & Togni, P. H. B. (2024). Untangling biodiversity interactions: A meta network on pollination in Earth's most diverse tropical savanna. *Ecology and Evolution*, *14*(3). https://doi.org/10.1002/ece3.11094

Alignier, A., Lenestour, N., Jeavons, E., van Baaren, J., Aviron, S., Uroy, L., Ricono, C., & Le Lann, C. (2023). Floral resource maps: a tool to explain flower-visiting insect abundance at multiple spatial scales. *Landscape Ecology*, *38*(6), 1511–1525. https://doi.org/10.1007/s10980-023-01643-9

Aminah, S. N., Nasruddin, A., Annisaa, N. W., Abdullah, T., & Fatahuddin. (2021). The presence of refugia and population of insect pest in rice field. *IOP Conference Series: Earth and Environmental Science*, *807*(2), 022093. https://doi.org/10.1088/1755-1315/807/2/022093

Aminatun, T., Budiwati, B., Rahmawati, Y. F., Widiyanti, D. N. A., & Fahdah, L. A. (2023). The effects of flowering trap crops on diversity and longevity of pollinator insect visitation on chili plants. *Biodiversitas Journal of Biological Diversity*, *24*(2). https://doi.org/10.13057/biodiv/d240226

Asmini, A., Atmowidi, T., & Kahono, S. (2022). Pollination by Three Species of Stingless Bees (Hymenoptera: Meliponini) Increase Seed Set of Mustard (Brassica rapa L.: Brassicaceae). *Hayati Journal of Biosciences*, *29*(5), 712–719. https://doi.org/10.4308/hjb.29.5.712-719

Bednaršek, N., Pelletier, G., Beck, M. W., Feely, R. A., Siegrist, Z., Kiefer, D., Davis, J., & Peabody, B. (2024). Predictable patterns within the kelp forest can indirectly create temporary refugia from ocean acidification. *Science of The Total Environment*, *945*, 174065. https://doi.org/10.1016/j.scitotenv.2024.174065

Boon, J. S., Keith, S. A., Exton, D. A., & Field, R. (2023). The role of refuges in biological invasions: A systematic review. *Global Ecology and Biogeography*, *32*(8), 1244–1271. https://doi.org/10.1111/geb.13701

Borror, D. J. , T. C. A. , & J. N. F. (1992). *An introduction to the study of insects (6th ed.). Saunders College Publishing.*

Bottero, I., Dominik, C., Schweiger, O., Albrecht, M., Attridge, E., Brown, M. J. F., Cini, E., Costa, C., De la Rúa, P., de Miranda, J. R., Di Prisco, G., Dzul Uuh, D., Hodge, S., Ivarsson, K., Knauer, A. C., Klein, A.-M., Mänd, M., Martínez-López, V., Medrzycki, P., … Stout, J. C. (2023). Impact of landscape configuration and composition on pollinator communities across different European biogeographic regions. *Frontiers in Ecology and Evolution*, *11*. https://doi.org/10.3389/fevo.2023.1128228

Burgio, G., Dindo, M. L., Pape, T., Whitmore, D., & Sommaggio, D. (2025). Diptera as predators in biological control: applications and future perspectives. *BioControl*, *70*(1), 1–17. https://doi.org/10.1007/s10526-024-10281-2

Callaway, B., Goodman-Bacon, A., & Sant'Anna, P. H. (2024). *Difference-in-differences with a Continuous Treatment*. https://doi.org/10.3386/w32117

Chellam, S., Bai, D. G., Rani, D. V., Sindhu, M., Pushpalatha, V., JS, R., & Manoosha, G. (2024). Agro-ecological approaches to pest management: The role of crop diversification and habitat manipulation. *International Journal of Advanced Biochemistry Research*, *8*(9S), 154–157. https://doi.org/10.33545/26174693.2024.v8.i9Sb.2077

Cohen, H., Philpott, S. M., Liere, H., Lin, B. B., & Jha, S. (2021). The relationship between pollinator community and pollination services is mediated by floral abundance in urban landscapes. *Urban Ecosystems*, *24*(2), 275–290. https://doi.org/10.1007/s11252-020-01024-z

David, M. A., Yommi, A., Sánchez, E., Martinez, A., Murillo, N., Marcellán, O., Atela, O., & Palacio, M. A. (2022). Strategic use of honey bees (Apis mellifera L.) to increase the number and size of fruits in kiwifruit (Actinidia chinensis var. deliciosa). *European Journal of Agronomy*, *133*, 126420. https://doi.org/10.1016/j.eja.2021.126420

Fried, G., Le Corre, V., Rakotoson, T., Buchmann, J., Germain, T., Gounon, R., Royer, H., Biju-Duval, L., Felten, E., Vieren, E., & Chauvel, B. (2022). Impact of new management practices on arable and field margin plant communities in sunflower, with an emphasis on the abundance of *Ambrosia artemisiifolia* (Asteraceae). *Weed Research*, *62*(2), 134–148. https://doi.org/10.1111/wre.12522

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., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N. P., Dudenhöffer, J. H., Freitas, B. M., Ghazoul, J., Greenleaf, S., … Klein, A. M. (2013). Wild Pollinators Enhance Fruit Set of Crops Regardless of Honey Bee Abundance. *Science*, *339*(6127), 1608–1611. https://doi.org/10.1126/science.1230200

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, *347*(6229). https://doi.org/10.1126/science.1255957

Guignard, Q., Spaethe, J., Slippers, B., Strube-Bloss, M., & Allison, J. D. (2021). Evidence for UV-green dichromacy in the basal hymenopteran Sirex noctilio (Siricidae). *Scientific Reports*, *11*(1), 15601. https://doi.org/10.1038/s41598-021-95107-2 Map of Research Location in Sumbersekar Village, Dau District, Malang Regency, East Java

Hagen, M., & Kraemer, M. (2010). Agricultural surroundings support flower–visitor networks in an Afrotropical rain forest. *Biological Conservation*, *143*(7), 1654–1663. https://doi.org/10.1016/j.biocon.2010.03.036

Hasriyanty, Yunus, M., Rosmini, Adelia, & Suriani. (2023). *Arthropoda Diversity in Paddy Field with Refugia Plants* (pp. 34–39). https://doi.org/10.2991/978-94-6463-334-4_7

Hederström, V., Ekroos, J., Friberg, M., Krausl, T., Opedal, Ø. H., Persson, A. S., Petrén, H., Quan, Y., Smith, H. G., & Clough, Y. (2024). Pollinator-mediated effects of landscape-scale land use on grassland plant community composition and ecosystem functioning – seven hypotheses. *Biological Reviews*, *99*(3), 675–698. https://doi.org/10.1111/brv.13040

Hou, Z., Liu, J., Cai, M., Liu, Y., Zhang, M., Wang, L., Yang, W., & Huang, B. (2023). The volatile organic compounds and palatability of mixed ensilage of marigold (Tagetes erecta L.) crop residues. *Scientific Reports*, *13*(1), 2080. https://doi.org/10.1038/s41598-023-28511-5

Jiang, S., Li, H., & Wu, K. (2023). Predation and Control Effect of Eupeodes corollae Fabricius (Diptera: Syrphidae) on Leguminous Plant Aphids. *Agronomy*, *13*(7), 1739. https://doi.org/10.3390/agronomy13071739

Jordan, A., Patch, H. M., Grozinger, C. M., & Khanna, V. (2021). Economic Dependence and Vulnerability of United States Agricultural Sector on Insect-Mediated Pollination Service. *Environmental Science & Technology*, *55*(4), 2243–2253. https://doi.org/10.1021/acs.est.0c04786

Jordão, J. P., da Silva, A. P., Nana, H. R. T., da Costa Pereira, R. R., & Fávaro, C. F. (2024). Ecology of entomological communities in cocoa flowers (Theobroma cacao L.) in the shade-grown system: harmonic interactions in pollination. *Agroforestry Systems*, *98*(8), 3179–3194. https://doi.org/10.1007/s10457-024-01082-8 Map of Research Location in Sumbersekar Village, Dau District, Malang Regency, East Java

Josephrajkumar, A., Mani, M., Anes, K. M., & Mohan, C. (2022). Ecological Engineering in Pest Management in Horticultural and Agricultural Crops. In *Trends in Horticultural Entomology* (pp. 123–155). Springer Nature Singapore. https://doi.org/10.1007/978-981-19-0343-4_4 Map of Research Location in Sumbersekar Village, Dau District, Malang Regency, East Java

Kumar, R., Hajam, Y. A., Kumar, I., & Neelam. (2024). Insect Pollinators's Diversity in the Himalayan Region: Their Role in Agriculture and Sustainable Development. In *Role of Science and Technology for Sustainable Future* (pp. 243–276). Springer Nature Singapore. https://doi.org/10.1007/978-981-97-0710-2_16

Kuppler, J., & Kotowska, M. M. (2021). A meta-analysis of responses in floral traits and flower–visitor interactions to water deficit. *Global Change Biology*, *27*(13), 3095–3108. https://doi.org/10.1111/gcb.15621

Lowe, E. B., Groves, R., & Gratton, C. (2021). Impacts of field-edge flower plantings on pollinator conservation and ecosystem service delivery – A meta-analysis. *Agriculture, Ecosystems & Environment*, *310*, 107290. https://doi.org/10.1016/j.agee.2020.107290

Loy, X., & Brosi, B. J. (2022). The effects of pollinator diversity on pollination function. *Ecology*, *103*(4). https://doi.org/10.1002/ecy.3631

Magurran AE. (2004). *Measuring Biological Diversity. Blackwell Scientific Oxford, UK.*

Maia-Silva, C., Hrncir, M., Giannini, T. C., Toledo-Hernández, M., & Imperatriz-Fonseca, V. L. (2024). Small Amazonian stingless bees: an opportunity for targeted cocoa pollination. *Frontiers in Bee Science*, *2*. https://doi.org/10.3389/frbee.2024.1357811

Michener, C. D. (2007). *. The bees of the world, 2nd edn. – Johns Hopkins University Press.*

Nath, R., Singh, H., & Mukherjee, S. (2023). Insect pollinators decline: an emerging concern of Anthropocene epoch. *Journal of Apicultural Research*, *62*(1), 23–38. https://doi.org/10.1080/00218839.2022.2088931

Noss, R. F., Cartwright, J. M., Estes, D., Witsell, T., Elliott, K. G., Adams, D. S., Albrecht, M. A., Boyles, R., Comer, P. J., Doffitt, C., Faber-Langendoen, D., Hill, J. G., Hunter, W. C., Knapp, W. M., Marshall, M., Pyne, M., Singhurst, J. R., Tracey, C., Walck, J. L., & Weakley, A. (2021). *Science needs of southeastern grassland species of conservation concern: A framework for species status assessments*. https://doi.org/10.3133/ofr20211047

Phan, N. T., Biddinger, D. J., Rajotte, E. G., Smagghe, G., Reddy, G. V., Ren, Z., & Joshi, N. K. (2025). Pesticide use in integrated pest and pollinator management framework to protect pollinator health. *Pest Management Science*, *81*(4), 1691–1696. https://doi.org/10.1002/ps.8582

Pioltelli, E., Guzzetti, L., Biella, P., Sala, D., Copetta, A., Mussano, P., Galimberti, A., & Labra, M. (2024). Animal pollination shapes fruits market features, seeds functional traits and modulates their chemistry. *Scientific Reports*, *14*(1), 22734. https://doi.org/10.1038/s41598-024-73647-7

Puttha, R., Venkatachalam, K., Hanpakdeesakul, S., Wongsa, J., Parametthanuwat, T., Srean, P., Pakeechai, K., & Charoenphun, N. (2023). Exploring the Potential of Sunflowers: Agronomy, Applications, and Opportunities within Bio-Circular-Green Economy. *Horticulturae*, *9*(10), 1079. https://doi.org/10.3390/horticulturae9101079

Rahmawati, Y. F. , & A. L. N. F. (n.d.). Effectiveness of Predator Diversity in Refuge Plants in Reducing Bactrocera sp. Population on Batu 55 Tangerines. *LenteraBio: Berkala Ilmiah Biologi*, *15*(1), 1–8.

Rahmawati, Y. F., Leksono, A. S., Gama, Z. P., & Rizali, A. (2025). The impact of refuges on Citrus orchards associated with arthropods in different agroecosystem in Malang, Indonesia. *Cogent Food & Agriculture*, *11*(1). https://doi.org/10.1080/23311932.2024.2448599

Rahmawati, Y. F., Leksono, A. S., Rizali, A., & Gama, Z. P. (2024). Population dynamics of Bactrocera spp., and parasitization efficacy of Opius sp. (Hymenoptera: Braconidae) in citrus varieties. *Biodiversitas Journal of Biological Diversity*, *25*(11). https://doi.org/10.13057/biodiv/d251134

Serralta-Batun, L. P. , J.-O. J. J. , M.-R. V. , & M.-R. M. A. (2024). Taxonomic and functional diversity of bees in traditional agroecosystems and tropical forest patches on the Yucatan Peninsula. *Tropical Conservation Science*, *17*(19400829231225428).

Setyadin, Y., Leksono, A. S., & Tarno, H. (2025). Diversity and bioindicators of natural enemies in organic paddy fields implementing habitat modification. *BIO Web of Conferences*, *154*, 01004. https://doi.org/10.1051/bioconf/202515401004

Shpak, Y. V., Leheta, U. V., & Sosnovskyi, K. S. (2023). Importance of pollinator diversity for sunflower seed production. *Agrology*, *6*(4), 79–85. https://doi.org/10.32819/021113 Map of Research Location in Sumbersekar Village, Dau District, Malang Regency, East Java

Singh, G., Cook, J. M., Spooner-Hart, R. N., & Makinson, J. C. (2025). Insect floral visitors vary spatiotemporally and influence fruit production in mango orchards. *Ecological Entomology*, *50*(6), 1121–1136. https://doi.org/10.1111/een.70005 Map of Research Location in Sumbersekar Village, Dau District, Malang Regency, East Java

Sladonja, B., Tlak Gajger, I., Uzelac, M., Poljuha, D., Garau, C., Landeka, N., Barták, M., & Bacaro, G. (2023). The Impact of Beehive Proximity, Human Activity and Agricultural Intensity on Diptera Diversity in a Mediterranean Mosaic of Agroecosystems, with a Focus on Pest Species. *Animals*, *13*(6), 1024. https://doi.org/10.3390/ani13061024

Sponsler, D., Iverson, A., & Steffan-Dewenter, I. (2023). Pollinator competition and the structure of floral resources. *Ecography*, *2023*(9). https://doi.org/10.1111/ecog.06651

Torezan-Silingardi, H. M., Silberbauer-Gottsberger, I., & Gottsberger, G. (2021). Pollination Ecology: Natural History, Perspectives and Future Directions. In *Plant-Animal Interactions* (pp. 119–174). Springer International Publishing. https://doi.org/10.1007/978-3-030-66877-8_6

Traut, W., Sahara, K., & ffrench-Constant, R. H. (2023). Lepidopteran Synteny Units reveal deep chromosomal conservation in butterflies and moths. *G3: Genes, Genomes, Genetics*, *13*(8). https://doi.org/10.1093/g3journal/jkad134

Ulyshen, M., Urban-Mead, K. R., Dorey, J. B., & Rivers, J. W. (2023). Forests are critically important to global pollinator diversity and enhance pollination in adjacent crops. *Biological Reviews*, *98*(4), 1118–1141. https://doi.org/10.1111/brv.12947

Vanlalhmangaiha, R., Singh, H. K., Boopathi, T., Lalhruaitluangi, S., & Sangma, T. T. (2023). Impact of insect pollination on the quantitative and qualitative characteristics of sweet orange, *Citrus sinensis* (L.) Osbeck. *Journal of Apicultural Research*, *62*(4), 767–776. https://doi.org/10.1080/00218839.2021.2013401

Villagómez, G. N., Spaethe, J., & Leonhardt, S. D. (2024). The stingless bee Trigona fulviventris prefers sweet and salty over savory nectar. *Apidologie*, *55*(4), 39. https://doi.org/10.1007/s13592-024-01081-9

Windriyanti, W., Rahmadhini, N., Fernando, I., & Kusuma, R. M. (2023). Arthropods discovered on refugio flowering plants in Mangifera indica plantation. *Biodiversitas Journal of Biological Diversity*, *24*(9). https://doi.org/10.13057/biodiv/d240915

Xerces Society for Invertebrate Conservation. (2021). *Farming with native beneficial insects: Ecological pest control solutions.* Storey Publishing.

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