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Effectiveness of Predator Diversity in Refuge Plants in Reducing *Bactrocera* sp. Population on Batu 55 Tangerines

Yunita Fera Rahmawati[1]*, Suliana[2], Nurul Fatihah Abd Latip[3]
[1]Department of Biology Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta, 55281, Indonesia
[2]Agricultural Extension Office (BPP), Dau District, Malang Regency, East Java 65151, Indonesia
[3]Faculty of Plantation and Agrotechnology, Universiti Teknologi Mara, Perlis Branch, Arau Campus, 02600 Arau, Perlis Malaysia
*Corresponding Author, e-mail: yunita.fr@uny.ac.id

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Abstract
Fruit flies (*Bactrocera* sp.) are a major pest of citrus fruits that can reduce the quality and yield of crops. Long-term use of chemical insecticides can damage the environment and endanger human health, so safer and more sustainable control methods are needed. This study aims to determine how effective flowering plants in a refugia system are in increasing predator diversity and controlling the population of *Bactrocera* sp. The study was conducted in the Batu 55 orange orchard by comparing three types of refugia plants, namely sunflowers (*Helianthus annuus*), marigolds (*Tagetes erecta*), and wild cosmos flowers (*Cosmos caudatus*), with a control group without refugia plants. The Shannon–Wiener diversity index and one-way anova test showed that flowering plants in the refugia system had a significant effect on increasing the predator population and reducing the number of *Bactrocera* sp. Sunflowers showed the best results, as they were able to increase the number of predatory insects by up to 163% and reduce the presence of fruit flies by approximately 40.7% compared to the control. The dominant predator species obtained in the tangerines orchard were *Coccinella* sp., *Cryptolaemus montrouzieri*, *Harmonia axyridis*, and *Creobroter gemmatus*. The results of this study confirm that the application of refugia involving flowering plants, mainly sunflowers, can increase predator diversity, maintain the balance of the citrus ecosystem, and support environmentally friendly and effective integrated pest management.

Keywords: Agroecosystem; biodiversity; insect predators; natural enemies; refuge.
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INTRODUCTION

Fruit flies (*Bactrocera* sp.) are among the most destructive pests in citrus production worldwide, causing losses of 30–80% (Abd-Elgawad, 2021), including in Batu 55 tangerines orchards in Dau District, Malang Regency. Infestations of this pest cause direct fruit damage, lower yields, and reduced market quality, leading to significant economic losses for farmers. The reliance of most traditional farmers on chemical insecticides to manage this pest raises serious concerns regarding human health, environmental safety, and the development of pest resistance (Pathak *et al*., 2022). This highlights the urgent need for sustainable and environmentally friendly pest management strategies that can be effectively implemented in citrus agroecosystems (Jain *et al*., 2023).

The implementation of a refugia system is an attractive solution that deserves continued development because it supports the arrival of natural enemies for pests, particularly fruit flies. These refugia provide broader ecological benefits by providing new habitats, additional food sources, and the availability of important prey for predatory insects. The development of these refugia aligns with the principles of Integrated Pest Management (IPM), which maintains the function of natural enemies as biological control agents, thereby reducing pest populations through the presence of beneficial arthropod species (Josephrajkumar *et al*., 2022; Rahmawati *et al*., 2025; Setyadin *et al*., 2025). Furthermore, this approach can also reduce dependence on chemical pesticides routinely used in research locations.

Some of the negative impacts of unwise pesticide use include air, water, and soil pollution, the killing of non-target organisms due to direct or indirect exposure, and the killing of non-target organisms (natural enemies) due to the ingestion of pesticides into the bodies of non-target insects. The accumulation of pesticides in organisms' tissues can lead to negative effects on humans, including resistance, resurgence, secondary pest outbreaks, eliminating natural enemies, high production costs, and obstacles to exporting agricultural products due to high pesticide levels (Saroop *et al.*, 2024).

Predator species play a significant role in preying on various life stages of fruit flies, including eggs, larvae, and adults. Therefore, it is hoped that effective control of their presence can be achieved (Bhagarathi and Maharaj, 2023). Natural enemies (parasitoids and predators) can naturally control pests that attack citrus fruits at all stages of life when the environment is conducive to their reproduction. This is possible because Indonesia's tropical agricultural ecosystem is conducive to the development of various types of natural enemies that can effectively control or suppress pest populations. However, inappropriate and environmentally unsound agricultural management practices, such as the irresponsible use of pesticides, can lead to the killing of existing natural enemies.

Flowering plants, such as wild cosmos (*Cosmos caudatus*), marigolds (*Tagetes erecta*), and sunflowers (*Helianthus annuus*), have morphological and physiological characteristics, including color, shape, size, fragrance, flowering period, and nectar content, that attract predatory insects. These insects are attracted to flowers with a long flowering period, which in turn attracts predatory insects such as beetles, weevils, and hoverflies, which play a significant role in suppressing the presence of *Bactrocera* sp. (Rahmawati *et al.*, 2024; Shaw *et al.*, 2021). The presence of natural enemies is central to biological control. Natural enemies present in an ecosystem, both native and exotic, are highly effective in suppressing existing pest populations. Agricultural ecosystems are simple and monoculture ecosystems, considering their community, vegetation selection, species diversity, and the risk of pest and disease outbreaks. Natural enemies play a role in reducing pest populations to non-harmful levels.

Research on refugia in horticultural crops is still limited, and further studies on predator diversity have not been widely conducted in Indonesia. This investigation has the specific objective of analyzing the diversity and abundance of arthropods, particularly predators, in Batu 55 tangerines orchards between plants with and without refugia. Furthermore, this research will help understand the effect of refugia on insect diversity and pest suppression levels. Furthermore, it is hoped that this citrus agroecosystem will further increase production, maintain ecological balance, and provide a scientific basis for pest control applications.

MATERIALS AND METHODS

The research was conducted in the hamlet of Banjar Tengah, Dau District, Malang Regency at the geographical coordinates of 7.916998° N and 112.569278° E. The Batu 55 tangerine variety (*Citrus reticulata* Blanco) was selected based on the tree's two- to four-year age that is in the active production phase (Rahmawati *et al*., 2024).

The research location was purposively selected in tangerines orchards, an area known as one of the main citrus production centers with high fruit fly infestation levels, representing typical highland agroecosystems with refuge habitat blocks. The research focused on two types of orchards: orchards with refuge and control orchards without refuge. The three types of refuge plants, sunflower (*Helianthus annuus*), marigold (*Tagetes erecta*), and wild cosmos (*Cosmos caudatus*), were planted intermittently with a distance between types of 0.65 meters, and one citrus tree in the middle of the garden as the center point of observation (Figure 1). Observations of the presence of predatory insects were made every two weeks from the flowering phase until harvest, with 24 replications.

Each observation session was conducted in three periods: 07:00-09:00 AM, 11:00 AM-01:00 PM, and 03:00-05:00 PM. Observations were conducted for 10 minutes on each citrus tree and each type of refuge plant, covering all plant parts with particular attention to flower-visiting insects as primary attractants for pollinators and natural enemies. Data collection was done through captures using insect nets and visual documentation. Insects identified directly in the field were recorded, while unidentified species were collected and taken to the Animal Diversity Laboratory for further analysis. Specimens were then preserved at the Brawijaya University Insectarium. The identification process was carried out to the family, genus, and species level using online references such as BugGuide and GBIF and literature such as Borror and DeLong's Introduction to the Study of Insects (Villet, 2005).

This study used a modified Steiner-type trap design to monitor the fruit fly population (Figure 2). This trap was made from an old plastic bottle equipped with several 0.7 cm diameter inlets in the center of the bottle to allow fruit flies to enter. Inside the bottle, a cotton swab was suspended using a wire and treated with petrogenol as an attractant to attract fruit flies. To kill the trapped insects, the bottom of the bottle was filled with a mixture of distilled water and 1% formalin. The trap was hung using oiled raffia to prevent ants from climbing into the trap.

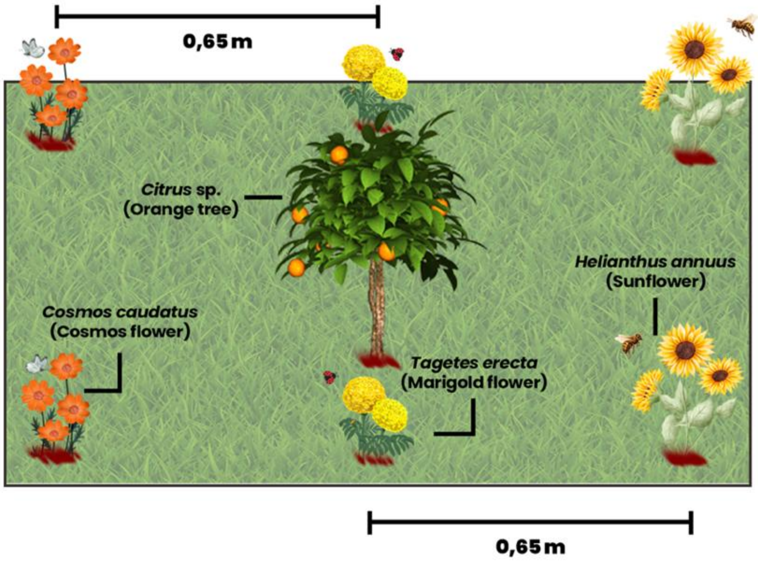

Figure 1. Citrus refuge plant design

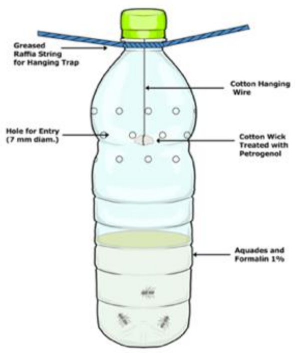

Figure 2. Design of modified Steiner-type fruit fly trap in Batu 55 tangerine orchard

Data analysis was performed using the Shannon-Wiener diversity index (H'), by calculating the proportion of each predator species (pi = ni/N), then multiplying it by the natural logarithm (ln pi), and summing it according to the formula $H' = -(pi \times \ln(pi))$. The H′ value obtained describes the level of species diversity in the community — the higher the H′ value, the higher the level of species diversity (Alcocer *et al.*, 2022).

RESULTS

The results of the inventory of predatory insects in the Batu 55 tangerines orchard, which implements a flowering refuge system, show a fairly high level of predator diversity and abundance. From this study, a total of 675 individuals consisting of 30 morphospecies, representing 10 orders and 19 families, were successfully identified. The highest number of individuals was recorded in sunflower refuge (262 individuals), followed by marigold (197 individuals), wild cosmos (114 individuals), and the control plots without refuge (102 individuals). The findings suggest that the placement of flowers as a refugia area is able to attract natural predators and increase the success of biological control in citrus orchards.

Compared to the other two refuge plants, sunflowers have been shown to invite the most predators, especially *Coccinella* sp., *Cryptolaemus montrouzieri*, *Harmonia axyridis*, and *Creobroter* *gemmatus*. Marigold also showed high attractiveness, dominated by *Coccinella* sp., *Creobroter gemmatus*, and *Tenodera angustipennis*, while wild cosmos was dominated by *Hippodamia moesta*, *Tenodera angustipennis*, and *Rodolia cardinalis*. Based on the Shannon-Wiener diversity index (H'), marigolds were recorded as having the highest diversity (2.14), followed by the wild cosmos (2.13), and sunflowers (2.03), while the control plot showed the lowest value (1.87). These results suggest that although sunflowers attracted the highest number of predators, marigold and wild cosmos refuge supported more diverse and balanced predator communities, whereas plots without refuge exhibited the lowest predator activity (Table 1).

Among the three types of refuge used, sunflower was the most attractive plant to predators, particularly *Coccinella* sp., *Cryptolaemus montrouzieri*, *Harmonia axyridis*, and *Creobroter gemmatus*. Marigold also showed high attractiveness, dominated by *Coccinella* sp., *Creobroter gemmatus*, and *Tenodera angustipennis*, while wild cosmos was dominated by *Hippodamia moesta*, *Tenodera angustipennis*, and *Rodolia cardinalis*. The Shannon–Wiener diversity index (H') indicated that marigolds had the highest diversity (H' = 2.14), followed by wild cosmos (H' = 2.13), sunflowers (H' = 2.03), and the control plot (H' = 1.87). These results suggest that although sunflowers attracted the highest number of predators, marigold and wild cosmos refuge supported more diverse and balanced predator communities, whereas plots without refuge exhibited the lowest predator activity.

Table 1. Abundance of predator species in Batu Tangerine 55

Species	Common name	Family	Treatments (with refuge)			Controls
			Sun-flower	Marigold	Wild cosmos	
Araneae						
Pisaurina sp.	Nursery web pider	Pisauridae	-	10	-	-
Oxyopes javanus (Thorell, 1887)	Striped lynx spider	Oxyopidae	1	2	1	1
Synalus angustus (L.Koch, 1876)	Narrow crab spider	Theridiidae	-	3	1	-
Neottiura bimaculata (Linnaeus, 1767)	True bugs	Miridae	-	2	-	-
Misumena vatia (Clerck, 1758)	Goldenrod crab spider	Theridiidae	-	2	-	-
Oxyopes gracilipes (White, 1849)	Graceful-legs Lynx	Oxyopidae	-	-	1	-
Mopsus mormon (Karsch, 1878)	Green jumping spider	Salticidae	-	1	-	-
Peucetia viridans (Hentz, 1845)	Green Lynx spiders	Oxyopidae	1	-	-	-
Coleoptera						
Coccinela sp. (Linnaeus, 1758)	Ladybird	Coccinellidae	80	69	13	36
Cryptolaemus montrouzieri (Mulsant, 1850)	Mealybug ladybird	Coccinellidae	47	12	10	30
Hippodamia moesta (LeConte, 1854)	Sorrowful lady beetle	Coccinellidae	15	16	28	9
Harmonia axyridis (Pallas, 1773)	Simply asian ladybeetle	Coccinellidae	46	5	4	7
Coccinella transversalis (Fabricus, 1781)	Small transverse ladybird beetle	Coccinellidae	21	3	2	3
Rodolia cardinalis (Mulsant, 1850)	Vedelia beetle	Coccinellidae	5	3	5	2
Coccinella septempunctata (Linnaeus, 1758)	Seven-spot ladybird	Coccinellidae	-	6	6	-
Chrysoperla rufilabris (Burmeister, 1839)	Red-lipped green lacewing	Chrysomelidae	3	2	-	-
Paroplapoderus sp.	Leaf-cutting weevil	Attelabidae	1	-	1	-
Dermaptera						
Forficula auricularia (Linnaeus, 1758)	European earwig	Forficulidae	6	-	-	-
Diptera						
Episyrphus balteatus (De Geer, 1776)	Marmalade hoverfly	Syrphidae	2	3	2	4
Toxomerus marginatus (Say, 1823)	Margined calligrapher fly	Syrphidae	-	-	2	-
Hemiptera						
Geocoris sp.	Bigeyed bugs	Geocoridae	1	-	6	2
Isyndus obscurus (Dallas, 1850)	Japanese assassin bug	Reduviidae	-	1	2	-
Mantodea						
Creobroter gemmatus (Saussure, 1869)	Jeweled flower mantis	Hymenopodidae	25	20	1	1
Tenodera angustipennis (Saussure, 1871)	Narrow-winged mantis	Mantidae	3	20	24	3
Neuroptera						
Chrysoperla carnea (Stephens, 1836)	Green Lacewing	Chrysopidae	-	1	-	-
Odonata						
Orthetrum sabina (Drury, 1770)	Slender skimmer	Libellulidae	4	5	2	1
Platycnemis latipes (Rambur, 1842)	White Featherleg	Platycnemididae	1	3	-	-

Species	Common name	Family	Treatments (with refuge)			Controls
			Sun-flower	Marigold	Wild cosmos	
Crocothemis erythraea (Brullé, 1832)	Scarlet dragonfly	Libellulidae	-	3	-	1
Pantala flavescens (Fabricius, 1798)	Globe skimmer	Libellulidae	-	3	2	2
Orthoptera						
Scolothrips sexmaculantus (Pergande, 1890)	Six-spotted thrips	Thripidae	-	2	1	-
Total			262	197	114	102
Diversity index (H')			2.03	2.14	2.13	1.87

Predator diversity was also reflected in the important role of the order Araneae, which ranked second in abundance and comprised the families Oxyopidae, Theridiidae, Pisauridae, and Salticidae. Species such as *Pisaurina* sp., *Synalus angustus*, and *Misumena vatia* were recorded as being highly active in wild cosmos and marigold refuge. Similarly, the presence of predators of the order Neuroptera (such as *Chrysoperla carnea* and *C. rufilabris*), Mantodea (*Creobroter gemmatus*, *Tenodera angustipennis*), and Diptera (*Episyrphus balteatus*, *Toxomerus marginatus*) were more often found in plots with refugia plants than in control plots. The predator community in the protected area is dominated by *Coccinella* sp., with a diversity index value (H') of 2.48, which is higher than that of the control plots (H' = 1.87). This indicates that the protection plot has a more stable, diverse, and balanced predator community structure.

The results of the one-way anova follow-up test showed that refugia had a highly significant effect on predator abundance ($F = 408.46$; $p = 4.34 \times 10^{-9} < 0.05$). Sunflower refugia proved to be the most effective treatment. The number of predators increased by 163% on sunflowers, 98% on marigolds, and 16% on wild cosmos. These results demonstrate that the use of refugia, particularly sunflowers, can significantly enhance the number and diversity of natural predators in citrus orchards. This method provides a sustainable solution for controlling fruit flies, a major pest.

Planting refuge plants can significantly reduce the number of fruit flies (*Bactrocera* sp.) and increase the populations of their predators. In 2023, only 1,835 fruit flies were found in the refuge area, compared to 3,351 in the control area. The largest decrease occurred during the fruiting season, particularly in February (111 in the refuge and 329 in the control), May (111 and 329), and July (383 and 525). The anova test showed that the refuge area significantly reduced the number of fruit flies ($F_{(count)} = 6.13$; $p = 0.0215 < 0.05$), with an average reduction of 40.7%. Table 2 shows that refugia were most effective in November (71.2%), February (66.3%), and April (68.7%), consistently reducing the number of fruit flies during the fruiting season.

This report demonstrates that the use of refugia enhances the diversity and abundance of natural predators in citrus orchards, while reducing the population of fruit flies. In this study, the planting of sunflowers, marigolds, and wild cosmos together helped maintain ecosystem balance and improve biological control in the Batu 55 mandarin orange orchard.

Table 2. Abundance of *Bactrocera* sp. Species in Batu Tangerine Orchards 55.

Months	Treatments (with refuge)/individuals	Controls/individuals
January	84	185
February	111	329
March	195	316
April	118	377
May	321	604
June	159	247
July	383	525
August	88	170
September	142	103
October	72	73
November	61	212
December	101	313
Total	1835	3351
	F = 6.13; p = 0.0215 (< 0.05)	

DISCUSSION

This study emphasizes the importance of applying refugia, especially in monoculture agricultural systems in the Batu 55 tangerines orchard, which shows high insect diversity and a sharp increase in fruit fly pests, especially during the pre-harvest period. Various studies on refugia that have discussed the use of wild plants in seasonal crops such as rice, corn, chili peppers, and others are interesting to continue developing in horticultural plant applications. This study's main objective is to analyze the diversity of natural enemies (especially predators) and evaluate efforts to suppress fruit fly populations.

The results of this study explain that sunflowers (*Helianthus annuus*) are the flowering plants most visited by predatory insects, followed by marigolds (*Tagetes erecta*) and wild cosmos (*Cosmos caudatus*). Several factors make predatory insects more attracted to sunflowers because they have large flower crowns that are more attractive to visit, abundant pollen, and a longer flowering time compared to the other two plants. This is in line with the statement by Jervis *et al*. (2023), who stated that flowers with abundant nectar will attract beneficial insects.

The types of insects that are most commonly found on sunflowers are *Coccinella* sp., *Cryptolaemus montrouzieri*, *Harmonia axyridis*, and *Creobroter gemmatus*. Sunflowers provide food for these species because they are a potential source of aphids, honeydew, and mealybugs, which satisfy their predatory hunting activities. The most common insects found on marigold flowers are *C. gemmatus* and *Tenodera angustipennis* because the scent emitted by marigolds attracts these two species to search for food. Meanwhile, *Hippodamia moesta* and *Rodolia cardinalis* are found on wild cosmos flowers because these species tend to prefer small insects from the Hemiptera order, whose arrival is also caused by chemical signals that make it easier for them to reach their food source. The differences between these three flowers prove that the shape of the flower, the size of the corolla, and the aromatic compounds emitted by the flowering plant increase predatory insects and their behavior in hunting for food (Ali *et al*., 2023).

The importance of flowering plants in this refugia mechanism supports the provision of shelter, food sources, and increases the diversity of predatory insects (Rytteri *et al*., 2021; Sentil *et al*., 2022). The arrival of these diverse types and numbers of predatory insects is crucial for naturally suppressing pest populations and maintaining the balance of agricultural ecosystems (Sofo *et al*., 2020). The role of these refugia blocks is crucial because these predatory insects can breed comfortably and feed on various types of pests, including *Bactrocera* sp. This suppresses the fruit fly population in citrus orchards. When these three types of flowering plants bloom simultaneously, the number of *Bactrocera* sp. significantly decreases. Various flowering plants have been shown to be helpful during the mating season by providing abundant food and safe egg-laying sites, thus supporting the reproductive process, especially in *Coccinelidae* sp.

The presence of other arthropods, such as spiders (Nyffeler and Gibbons, 2022) and predatory insects (*Chrysoperla* sp.), which feed on pest eggs, and *Episyrphus balteatus*, demonstrates the importance of flowering plants for natural pest control. These refugia systems provide additional food sources that predatory insects cannot obtain in their primary feeding locations. These three insects provide a clearer understanding that the application of this refugia system is a key element in controlling pest eggs and larvae (Pathrose *et al*., 2023). Meanwhile, the predator with the smallest number in the three flower series, *Platycnemis latipes*, a damselfly, is likely not a major predator on citrus plants and the available prey is less suitable. The diversity of predatory insects from various orders in this tangerines orchard location demonstrates that the refugia system fully supports the presence of these insects, increasing their abundance and diversity.

In addition to increasing the presence of predatory insects, the refugia system can also continuously suppress fruit fly pest populations over time. In the control treatment, the number of fruit fly individuals increased from January to December, while the refugia application was more stable and did not experience an explosion. The fluctuations were recorded as moderate, with a non-extreme increase occurring from May to July. Meanwhile, in October and November, a significant decrease was observed, reflecting the presence of habitat support in the citrus orchard. This demonstrates a complex relationship between predators and prey, further strengthening the analysis results at the tangerines orchard location with refugia ($F_{(count)} = 6.13$; $p = 0.0215 < 0.05$). There was an average reduction in fruit fly pests of 40.7%, right during fruit formation and flowering in citrus plants. Therefore, if the application of these refugia is carried out continuously with proper monitoring, the pest population will be suppressed.

Integrating refugia with natural enemy conservation efforts on site, by implementing refugia involving flowering plants, is crucial for creating a stable ecological balance (Duff *et al*., 2024; Rossetto & Kooyman, 2021). The application of sunflowers, marigolds, and wild cosmos has been shown to support the survival of predatory insects and suppress the presence of fruit flies in Batu 55 orange orchards. Ultimately, the continued use of chemical pesticides will kill non-target species, including predators. Therefore, the implementation of these environmentally friendly refugia requires serious attention to ensure continued citrus productivity increases. Various studies on the importance of these refugia are supported by numerous previous studies that underscore the importance of improving ecosystem function in achieving healthy agriculture and productive without reducing the harvest.

CONCLUSION

The results of this study indicate that the use of refugia has a significant effect on the presence of predatory insects in suppressing the fruit fly pest *Bactrocera* sp. The application of this refugia was able to reduce the presence of fruit flies by up to 40.7% throughout 2023. Sunflowers were the most effective flower plant because predator visits reached 163% compared to the other two flowers, namely marigolds at 98% and wild cosmos at 16%. *Coccinella* sp. beetles (80 individuals), *Cryptolaemus montrouzieri* (47 individuals), *Harmonia axyridis* (46 individuals), and *Creobroter gemmatus* (25 individuals are important species that play a role in suppressing the presence of fruit flies, but their specific roles need to be studied further. Based on the anova test, it was concluded that there was a significant difference in the application of refugia and the diversity of predator numbers ($F = 408.46$; $p = 4.34 \times 10^{-9}$) and a decrease in the population level of fruit flies ($F = 6.13$; $p = 0.0215$). Furthermore, the application of this refugia emphasizes the importance of maintaining ecosystem stability in citrus orchards as an integrated pest management attempt.

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CONFLICT OF INTEREST

There is no conflict of interest.

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