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Research Article

Olfactory Response of Diaphorina citri to Guava Leaves Powder

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ABSTRACT

Citrus Vein Phloem Degeneration (CVPD), transmitted by the *Diaphorina citri*, remains a major challenge to global citrus production. Vector management rely on chemical insecticides which are not environmentally friendly and deemed to be less effective. This study was conducted to identify the ability of dried guava leaf extract on reducing *D. citri* olfactory responses. Y-tube olfactory test was conducted to identify the repellency effect of a various proportion and leaf ages of citrus and guava (*Psidium guajava*) leaf extract extracts to insect vector and its predator (*Menochilus sexmaculatus*). Results showed that guava leaf extracts had repellent effects on *D. citri*, but not on *M. sexmaculatus*. The repellant effect was higher on young guava leaves than on medium and old ones. Red guava leaves had the highest repellant effects compared to white guava and non-seed guava leaves. Guava leaf extract is an alternative means to control *D. citri*. However, the types of compounds that function as repellants need to be studied further.

Keywords: CVPD; leaf extract; repellent; vector

INTRODUCTION

Between 2008 and 2012, Indonesian citrus production experienced its most significant decline by approximately 65.3%. This significant decline resulted in an increase in citrus fruit imports, rising from 138,000 tons worth of 117 million USD in 2008 to 256,000 tons valued at 247 million USD by 2012 (Nurhadi, 2015).

Citrus plants are vulnerable to pests and diseases that can severely impact their yield. A notable pest is *Diaphorina citri* (Hemiptera: Liviidae), which serves as a vector for Citrus Vein Phloem Degeneration (CVPD), also known as Huanglongbing (Hung *et al.*, 2004). CVPD inflictions lead to high mortality rates, reduced productive lifespan, and diminished fruit quality and yield. This disease is caused by Gramnegative bacteria, including *Candidatus Liberibacter asiaticus*, *Candidatus Liberibacter africanus*, and *Candidatus Liberibacter americanus* (Nakashima *et al.*, 1998; Teixeira *et al.*, 2005). Both adult Asian Citrus psyllids (*Diaphorina citri*) and their 4th instar nymphs are capable of transmitting the disease (Ammar *et al.*, 2020). While vectors can carry the disease throughout their lives, pathogens are also transovarial and occurs at a rate of 2-6% (Chen *et al.*, 2023).

In Indonesia, four primary strategies have been implemented to manage Citrus Vein Phloem Degeneration (CVPD): disease-free seeds, quarantine, destruction of infected plants, and insect vector management (Supriyanto & Whittle, 1991; Monzo & Stansly, 2017). Despite these efforts, the management of D. citri still heavily depends on synthetic chemical insecticides, which are costly and environmentally unsustainable (Monzo & Stansly, 2017). Mineral oil presents a potential alternative to these synthetic chemicals, though it is not frequently used and costly. Research has shown that mineral oils can interfere with D. citri's ability to detect volatile compounds emitted by citrus leaves, potentially causing confusion and failure to identify and find host plants. Use of mineral-based oils may reduce the emission of attractant volatiles, mask these attractants, release repellent volatiles, or repel D. citri through their

volatiles (Poerwanto *et al.*, 2012). Additionally, intercropping guava with citrus plants using a ratio of one guava plant for every two citrus plants has been found to decrease *D. citri* populations. However, this method does not prevent CVPD occurrences on citrus plants (Gottwald *et al.*, 2014).

Insects locate host plants primarily through their olfactory systems which detect plant-emitted volatile compounds. The response of insects to these odors depends on both the quality and quantity of the volatile organic compounds. Insect preference for specific host plants also contributes to their attractions (Conchou et al., 2019). Volatile organic compounds and secondary metabolites emitted by plants serve as significant cues for insects like D. citri in finding their hosts (Poerwanto et al., 2008; 2012). These compounds evaporate rapidly and vary by plant species, contributing to plant-specific attractiveness. Volatile compounds released by plants from their leaves, flowers, or fruits are essential in attracting insects. In addition to volatile organic compounds, the physical and chemical characteristics of the plants can also influence insect attraction. Diaphorina citri relies on specific volatile odors from citrus shoots that formic acid and acetic acid which are significant this species' host acceptance (George et al., 2016).

The volatile profile of *Psidium guajava* L. (guava) leaves has been the subject of significant research, revealing a diverse array of compounds. Lee et al. (2011) identified terpene hydrocarbons and C6 compounds as predominant contributors including α -pinene, β -caryophyllene, and α -humulene. Additionally, Sagrero-Nieves et al. (1994) highlighted δselinene, α -selinene, and α -caryophyllene as major components. Further investigations conducted by Sagrero-Nieves et al. (1994) and Xu et al. (2017) confirmed that sesquiterpenoids, including α -selinene, α -caryophyllene, and δ -selinene, were prevalent in guava leaf extracts. Despite these findings, the specific types and mechanisms of organic volatile responsible for the deterrence of D. citri remains elusive.

Research is needed to assess guava leaves effectiveness in repelling *D. citri* by examining its impact on the vector's olfactory response. Furthermore, examining the impact of guava leaves on the natural enemies of *D. citri*, such as *Menochilus sexmaculatus*, a known predator that can reduce *D. citri* populations by up to 90% in the absence of its primary prey, *Aphis craccivora* (Ramadhan *et al.*, 2008), is crucial for integrated pest management strategies. This research aims to determine the ability of dried guava leaves to diminish the olfactory attraction of *D. citri* through Y-tube olfactory tests, comparing the repellent effects of variously aged guava leaf extracts and their mixtures with citrus leaf extracts on both the vector and its predator.

MATERIALS AND METHODS

Research was done in the greenhouse and Laboratory of Plant Protection of the Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta. Rearing of *D. citri* was done in a greenhouse. Disease-free *D. citri* adults (psyllids) were sourced from the Research Centre for Citrus and Subtropical Fruit in Malang, Indonesia. *Murraya paniculata* (orange jasmine) plants were used as the host plants. These plants were grown in plastic pots (25 cm in diameter, 25 cm in height) and placed in plastic gauze cages (60 cm \times 60 cm \times 100 cm). Buds that emerged after pruning served as oviposition sites.

Rearing of Menochilus sexmaculatus

Ladybird beetles (*Menochilus sexmaculatus*) rearing was conducted using aphids as prey. Initially, aphids were cultured on long bean plants. The seedlings were grown in polybags (15 cm in diameter, 15 cm in height) and placed inside a cage covered with gauze (100 cm \times 100 cm \times 100 cm). Following a seven-day period, the leaves exhibited infestation by field-collected aphids. The long bean plants were substituted with fresh ones whenever they started to wilt or dry out due to aphid infestation. A mating pair of adult *M. sexmaculatus* was introduced into the gauze cage containing the long bean plants and aphids for egg-laying. The eggs hatched after 3 to 4 days, and adult beetles emerged after 14 to 15 days.

Leave Powder Preparation

This study utilized fully expanded citrus leaves (*Citrus reticulata*) and guava leaves (*Psidium guajava*). Young leaves (leaf positions 1 and 2 from the shoot),

medium leaves (leaf positions 3 and 4 from the shoot), and old leaves (leaf positions 5 and 6 from the shoot) of red guava, white guava, and seedless guava were oven-dried at 50 °C for 24 hours. After drying, the leaves were pulverized with an electric grinder and subsequently sieved to obtain fine powder and remove unwanted particles. The leaf powder was kept in sealed containers to preserve its quality.

Olfactory Test

An olfactory test was conducted to assess adult *D. citri* (5–7 days old) and *M. sexmaculatus* (7–14 days old) responds by employing an adapted Y-shaped olfactometer, consisting of a transparent glass tube with an internal diameter of 10 mm and a length of 300 mm (Poerwanto *et al.*, 2012). The primary tube was designated as A, with the left

branch labeled B and the right branch marked as C. Ten mixed-gender D. citri adults were introduced at the end of tube A. In airtight containers (150 mm \times 150 mm \times 150 mm), 5 g of guava leaf powder (based on treatment) was connected to branch B, while 5 g of citrus leaf powder was connected to branch C, both via silicone tubes (5 mm internal diameter). Air was purified by passing it through activated charcoal and distilled water to remove particles and add humidity. The purified air was then directed to both odor sources at a flow rate of 20 mL/min, assessed using an air flow meter. The test insects were given 60 minutes to choose between ends B and C, and their choices were recorded. This procedure was conducted 30 times, and the identical approach was used for adult M. sexmaculatus. The list of odor source comparisons is provided in Table 1.

Table 1. Treatment sets of Y-tube olfactometer assay conducted for 30 minutes with 10 adults of *Diaphorina citri* or *Menochilus sexmaculatus* against citrus leaves powder versus young, medium, and old leaves from citrus, red, white, and non-seed guava leaves powder

Comparison	Odor sources
Ι	5 g Ct Vs 5 g Ct + 5 g RGY
II	5 g Ct Vs $5 g Ct + 5 g WGY$
III	5 g Ct Vs $5 g Ct + 5 g NSGY$
IV	5 g Ct Vs $5 g Ct + 5 g RGM$
V	5 g Ct Vs $5 g Ct + 5 g WGM$
VI	5 g Ct Vs $5 g Ct + 5 g NSGM$
VII	5 g Ct Vs $5 g Ct + 5 g RGO$
VIII	5 g Ct Vs $5 g Ct + 5 g WGO$
IX	5 g Ct Vs $5 g Ct + 5 g NSGO$
X	5 g Ct + 5 g RGY Vs $5 g Ct + 5 g RGO$
XI	5 g Ct + 5 g RGM Vs $5 g Ct + 5 g RGO$
XII	5 g Ct + 5 g RGY Vs $5 g Ct + 5 g RGM$
XIII	5 g Ct + 5 g WGY Vs $5 g Ct + 5 g WGO$
XIV	5 g Ct + 5 g WGY Vs $5 g Ct + 5 g WGM$
XV	5 g Ct + 5 g WGM Vs $5 g Ct + 5 g WGO$
XVI	5 g Ct + 5 g NSGY Vs $5 g Ct + 5 g NSGO$
XVII	5 g Ct + 5 g NSGM Vs $5 g Ct + 5 g NSGO$
XVIII	5 g Ct + 5 g NSGY Vs $5 g Ct + 5 g NSGM$
XIX	15 g Ct Vs 7.5 g RGY + 7.5 g Ct
XX	15 g Ct Vs 5 g RGY + 10 g Ct (1:2)
XX1	15 g Ct Vs 10 g RGY + 5 g Ct (2:1)
XXII	15 g Ct Vs 15 g RGY
Note: Ct : Citrus leaves	WGM : White guava medium leaves
RGY: Red guava young leaves	WGO : White guava old leaves
RGM : Red guava medium leaves	NSGY : Non-seed guava young leaves
RGO : Red guava old leaves	NSGM : Non-seed guava medium leaves
WGY: White guava young leaves	NSGO : Non-seed guava old leaves

RESULTS AND DISCUSSION

Insects heavily depend on plants' volatile organic compounds (VOCs) to locate their host plants. Their response to these odors varies based on the plant part, with stronger responses observed from plant parts that are their primary food source (Nusra *et al.*, 2021). Each insect species utilizes specific groups of olfactory receptors, allowing them to adaptively respond to complex volatile compounds mixtures (Conchou *et al.*, 2019). Plants attractiveness that serve as hosts for insects is influenced by both the quality and quantity of emitted VOCs. Healthy plants are more appealing to insects for feeding and oviposition (Lin *et al.*, 2022).

Psyllids Respons to Red, White, and Non-Seed Guava Leaves Powder Odor

The olfactory response of adult *D. citri* (psyllids) was observed based on their movement towards odor sources. Fewer psyllids were attracted to the mixture of citrus leaves and guava leaves powder compared to citrus leaves alone. The odor from guava leaf powder significantly decreased the number of psyllids moving towards odor sources (P <0.001). The number of psyllids attracted to the citrus and guava leaf mixture were lower than those moving toward the citrus leaf odor alone. Some psyllids did not move at all during the 60-minute observation period. The mean number of psyllids $(\pm$ SE) that moved to citrus leaves, the mixture of citrus and young red guava leaves, and those that did not move were 7.00 \pm 0.32, 2.33 \pm 0.16, and 0.67 ± 0.23 , respectively (Table 2).

Similar results were found between middle-aged and old guava leaves, where most psyllids were attracted to the citrus leaf odor, with only a few moving towards guava leaf odors, and some remaining stationary. This pattern was consistent across red, white, and seedless guava. Psyllids' responses to odors from the different guava leaf types were similar.

These findings aligned with Zaka *et al.* (2010), who found that odors from crushed guava leaves diminished psyllid attraction. Similar effects were observed in intercropping experiments between guava and citrus (Gottwald *et al.*, 2014), where the population of *D. citri* was reduced in citrus intercropped with red guava, but not with white guava. Poerwanto and Solichah (2020) compared the repellency of red, white, and seedless guava leaf extracts on psyllids, finding that red guava had the highest repellency, followed by seedless guava, with white guava being the least effective. However, the differences in volatile organic compounds between these guava varieties remained unclear.

Host selection by *D. citri* is influenced by the presence of formic and acetic acid emitted by citrus leaves, which increase probing behavior when present (George *et al.*, 2016). It is suggested that guava leaf volatiles interfere with these stimuli, disrupting the psyllids' ability to locate their host plants.

Psyllids Response to Young, Middle, and Old Guava Leaves

Different responses of *D. citri* psyllids to young, middle, and old guava leaves were observed (Table 3). Old leaves were significantly more attractive than young leaves, with 3.07 ± 0.07 psyllids moving toward young leaves, compared to 3.53 ± 0.13 moving toward old leaves. When comparing middle-aged leaves to old leaves, more psyllids were attracted to the odor of old leaves (5.67 \pm 0.16), while only 2.13 \pm 0.19 moved to the middle-aged leaves. Similar results were found with white guava leaves: more psyllids were attracted to old leaves (6.07 ± 0.18) than young leaves (2.67 \pm 0.25). Additionally, old leaves were more preferred (4.67 \pm 0.25) than middle leaves (3.33 ± 0.25) , and middle leaves were preferred (5.33 ± 0.13) over young leaves (2.33 ± 0.33) . The same pattern was observed in seedless guava, where old leaves were generally the most attractive, followed by middle-aged leaves, with young leaves being the least attractive.

This suggests that the volatile compound content in guava leaves changes with age. There may also be differences in the composition of volatile compounds between young, middle, and old leaves. In eucalypts, the type and intensity of volatile emissions were species-specific and depended on plant tissues (Sørensen *et al.*, 2020). Similarly, the release of volatile organic compounds (VOCs) from apple and peach leaves gradually decreased as the plant develops (Li *et al.*, 2021). Corn leaves also showed varying levels of VOCs emissions at different stages of leaf development, with older leaves emitting lower intensities than younger ones (Mozaffar *et al.*, 2018).

1	0			
Leaf age -	Odor sources		No attraction	Р
	Citrus	Citrus + guava	ino attraction	Г
	Red	Guava		
Young	7.00 ± 0.32	2.33 ± 0.16	0.67 ± 0.23	< 0.001
Medium	7.20 ± 0.28	2.33 ± 0.16	0.53 ± 0.19	< 0.001
Old	7.80 ± 0.17	2.13 ± 0.19	0.07 ± 0.07	< 0.001
	White	e Guava		
Young	5.73 ± 0.36	2.73 ± 0.23	1.53 ± 0.46	< 0.001
Medium	6.00 ± 0.39	2.47 ± 0.17	1.52 ± 0.45	< 0.001
Old	6.47 ± 0.21	2.33 ± 0.16	1.20 ± 0.29	< 0.001
	Non-se	ed Guava		
Young	6.73 ± 0.25	2.73 ± 0.31	0.53 ± 0.24	< 0.001
Medium	6.93 ± 0.22	2.60 ± 0.29	0.47 ± 0.22	< 0.001

Table 2. Comparison of adult *Diaphorina citri* attraction to odour of citrus leaves compared to leaf extracts from different species and ages

Note: Means \pm SE followed by different lowercase letters within a row were significantly different at P < 0.05 according to Duncan's Multiple Range Test (N = 30).

 1.87 ± 0.26

Table 3. Number of adult Diaphorina citri attraction to odour from different guava varieties and ages

 7.67 ± 0.25

Variety of guava	Odor sources			- Not move	Р
valiety of guava	Young	Middle	Old		1
	3.07 ± 0.07 a	-	3.53 ± 0.13 b	3.40 ± 0.13 a	< 0.001
Red	-	2.13 ± 0.19 p	5.67 ± 0.16 q	2.20 ± 0.31 p	< 0.001
	$1.80\pm0.11~\mathrm{x}$	4.40 ± 0.26 y	-	3.80 ± 0.14 z	0.002
	2.67 ± 0.25 a	-	6.07 ± 0.18 b	1.33 ± 0.27 c	< 0.001
White	-	3.33 ± 0.25 p	$4.67 \pm 0.25 \text{ q}$	$2.00 \pm 0.44 \text{ r}$	< 0.001
	2.33 ±0.33 x	5.33 ± 0.13 y	-	$2.33 \pm 0.45 \text{ x}$	< 0.001
	2.67 ± 0.39 a	-	3.93 ± 0.23 b	3.40 ± 0.43 a	< 0.001
Non-seed	-	2.93 ± 0.27 p	$7.07 \pm 0.27 \text{ q}$	$0.00 \pm 0.00 \text{ r}$	< 0.001
	$4.47 \pm 0.19 \text{ x}$	5.07 ± 0.18 y	-	$0.47 \pm 0.17 \text{ z}$	< 0.001

Note: Means \pm SE followed by different lowercase letters within a row were significantly different at P < 0.05 according to Duncan's Multiple Range Test (N = 30).

It is suspected that sulfur-based volatile compounds, including dimethyl disulfide (DMDS) produced by guava leaves, play a role in reducing D. citri's attraction to host plant volatiles. However, other sulfur compounds, such as dipropyl disulfide, ethyl-1-propyl disulfide, and diethyl disulfide, did not reduce the attraction (Onagbola et al., 2011). DMDS was a volatile sulfur compound that peaks within 10 minutes of guava leaf damage but quickly decreased and degraded into other sulfur compounds (Rouseff et al., 2008). However, Silva et al. (2016) found that although guava oil extracts exhibited repellent properties against D. citri, none contained sulfur compounds. Their tests on oil extracts from immature and mature guava leaves showed that immature leaves were more attractive

Old

to *D. citri*. This indicated that other compounds beside DMDS may also have repelled properties, and their concentration inversely correlated with leaf age, being more abundant in older leaves.

 0.47 ± 0.22

Psyllids Response to Different Ratio of Mixture of Citrus Leaves and Guava Leaves

The ratio of citrus and guava leaf mixtures as odor sources significantly influenced the psyllids responses. The presence of guava leaf odor reduced psyllid attraction. A 1:1 ratio of guava to citrus leaf powder significantly decreased the number of psyllids moving toward the odor source (2.67 \pm 0.09), whereas most psyllids were attracted to the odor of citrus leaves alone (5.67 \pm 0.08). A small number (1.67 \pm 0.18) remained stationary, not responding to either the citrus or mixed odor source (Figure 1).

< 0.001

When the amount of citrus powder was doubled to a 1:2 ratio (guava:citrus), the number of psyllids attracted to the mixed odor increased (3.67 ± 0.09), matching the number attracted to citrus alone (Figure 2). However, increasing guava leaf ratio to 2:1 (guava:citrus) significantly reduced psyllid attraction to the mixture (2.33 ± 0.08), while 5.00 ± 0.26 psyllids still moved toward the citrus odor source. The number of psyllids that also did not move increased (2.67 ± 0.32) (Figure 3).

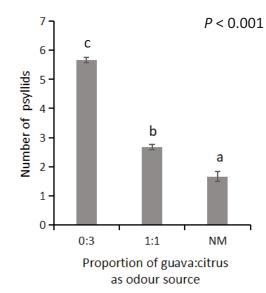
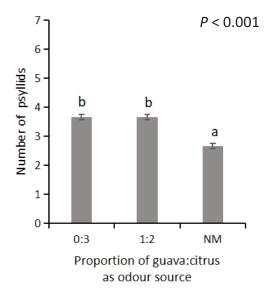
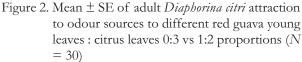


Figure 1. Mean ± SE of adult *Diaphorina citri* attraction to odour sources to different red guava young leaves : citrus leaves 0:3 vs 1:1 proportions (N = 30)





The response was even more pronounced when comparing pure citrus and guava odors without mixing (Figure 4). The number of immobile psyllids rose to 3.6 ± 0.10 , while fewer psyllids moved toward the citrus odor (4.17 ± 0.07), and the number attracted to the guava odor remained low (2.23 ± 0.11).

It is suggested that there may be allelopathic interactions between the volatile compounds of guava and citrus leaves in the air, reducing the host

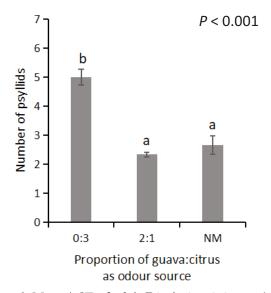
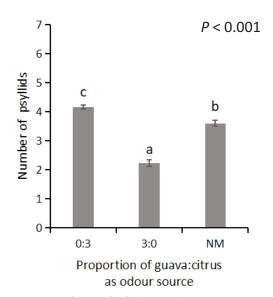
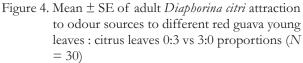


Figure 3. Mean ± SE of adult *Diaphorina citri* attraction to odour sources to different red guava young leaves : citrus leaves 0:3 vs 2:1 proportions (N = 30)





plant's attractiveness (Barman *et al.*, 2016). Another possibility is that guava volatiles masks or overshadows attractive volatiles from citrus leaves (Poerwanto *et al.*, 2012). The psyllid's attraction is influenced by the intensity of volatile emissions, with their response being dose-dependent (Onagbola *et al.*, 2011). Similar findings were observed when intercropping apple plants with aromatic plants, which reduced the attraction of *Lyonetia prunifoliella* by altering the insect's ability to recognize host plants through volatile signals (Huang *et al.*, 2020).

The optimal ratio that significantly reduces the number of psyllids can serve as a foundation for using guava plants in managing *Candidatus Liberibacter asiaticus* (CLas), which causes Citrus Vein Phloem Degeneration (CVPD). This approach can inform the number of guava trees required to be interplanted with citrus, as well as the appropriate dose or concentration of guava leaf extract to repel psyllids.

Ladybird Response to Guava Leaves

Guava leaves did not diminish the attraction of *M. sexmaculatus*. In fact, the number of ladybird responding to the odor of guava leaves was significantly greater than that to citrus leaves. Young, middle-aged, and old leaves of both red and white guava were markedly more appealing to ladybird compared to citrus leaves. The response to young and middle-aged of red guava leaves was notably

lower compared to the control, but the highest attraction was observed with old red guava leaves. Similarly, most adult predators reacted to the odor of young and middle-aged white guava leaves. However, old guava leaves elicited a different response, with most predators showing no reaction to their odor. A similar pattern was observed with non-seed guava leaves, where the odor was less attractive compared to citrus leaves (Table 4), and the majority of predators did not respond.

While existing research indicates that guava plants or compounds extracted from guava leaves can repel or reduce the attractiveness of *D. citri*, there is no current evidence regarding guava leaves' capacity to attract predatory insects targeting *D. citri*.

Plant volatiles, including those from non-host plants, can significantly impact the behavior of natural enemies and their prey (Glinwood *et al.*, 2009; Ninkovic & Åhman, 2009). Predators and parasitoids use these volatiles to locate the habitat and presence of their prey. For example, volatiles released by plants infested by herbivores serve as attractants for predators, such as the predatory mite *Neoseiulus longispinosus* which then attack the red spider mite *Oligonychus coffeae* (Rahman & Babu, 2021). Predators responses are influenced by the type and intensity of the volatile substances released by host plants. Variations in volatile intensity

Ass of lower	Odor sources		Not move	Р
Age of leaves -	Citrus	Citrus + guava	Not move	P
	Red	Guava		
Young	0.00 ± 0.00	4.80 ± 0.56	5.20 ± 0.56	< 0.001
Medium	1.20 ± 0.30	3.60 ± 0.28	5.20 ± 0.18	< 0.001
Old	1.20 ± 1.90	5.20 ± 0.18	3.60 ± 0.36	< 0.001
	White	e Guava		
Young	0.40 ± 0.15	5.60 ± 0.49	4.00 ± 0.53	< 0.001
Medium	0.80 ± 0.18	5.60 ± 0.28	3.60 ± 0.36	< 0.001
Old	1.60 ± 0.15	3.60 ± 0.43	4.80 ± 0.45	< 0.001
	Non-se	ed Guava		
Young	2.00 ± 0.23	0.40 ± 0.15	7.60 ± 0.15	< 0.001
Medium	1.60 ± 0.28	4.40 ± 0.36	4.00 ± 0.33	< 0.001
Old	3.20 ± 0.56	0.80 ± 0.14	6.00 ± 0.69	< 0.001

Table 4. Comparison of adult *Menochilus sexmaculatus* attraction to odour of citrus leaves compared to leaf extracts from different species and ages

Note: Means \pm SE followed by different lowercase letters within a row were significantly different at P < 0.05 according to Duncan's Multiple Range Test (N = 30).

among different tomato varieties have been shown to affect the attractiveness of *Nesidiocoris tenuis* to prey-infested plants (Abdollahipour *et al.*, 2020). Additionally, predators use plant volatiles as habitat markers to locate their prey. For instance, adults of *Harmonia axyridis* are more attracted to both healthy and aphid-infested plants compared to control plants (Cai *et al.*, 2020). Similarly, larval parasitoids of Lepidoptera, such as *Microplitis croceipes* and *Cotesia marginiventris*, rely on volatile cues from host plants to locate their prey (Das *et al.*, 2017). The parasitoid *Anagyrus dactylopii* has also shown increased attraction to a blend of volatile organic compounds from aromatic and apple plants (Huang *et al.*, 2020).

Guava leaf extract has potential as a management method for D. citri without diminishing the appeal to its natural predators. Management strategies for D. citri can be achieved through several approaches: utilizing the repellent properties of guava leaves, leveraging the predatory activities of M. sexmaculatus, a generalist predator, and enhancing repellent effects through the presence of these predators. Studies have shown that D. citri avoids plants with traces of the predator Hippodamia convergens for feeding and oviposition (Seo et al., 2018). Additionally, guava leaves may attract other predatory species, warranting further investigation into various predator species. Other potential biological controls include green lacewings such as Chrysoperla rufilabris and C. comanche, as well as the brown lacewing Sympherobius barberi, and coccinellids like Diomus pumilio and Rhyzobius lophanthae, all of which have been effective in reducing psyllid populations (Gómez-Marco et al., 2022). Observations by Rugno et al. (2021) revealed that lacewings including Ceraeochrysa cincta, Ceraeochrysa cubana, Ceraeochrysa paraguaria, Chrysoperla externa, and Chrysoperla defraetata were abundant in citrus orchards and thrive on D. citri nymphs. Additionally, planting insectary plants like Lobularia maritime and Fagopyrum esculentum, which produce plentiful flowers, can attract 10-20 times more syrphids such as Allorapta obliqua, leading to a 3.5-fold increase in egg laying and a 2.5-fold increase in predator abundance (Irvin et al., 2021). For optimal management of D. citri, integrating guava leaf extract with phytoene desaturase-silenced citrus as trap plants around orchards, which provide visual, olfactory, and gustatory cues, could enhance control efforts (Killiny *et al.*, 2021).

CONCLUSION

Guava leaves demonstrated repellent effects against *D. citri* but not against *M. sexmaculatus*. This repellent effects was more pronounced from young guava leaves compared to medium and older leaves. Red guava leaves have the highest repellant effects than white guava and non-seed guava leaves. Guava leaf extract was an alternative to manage *D. citri*. However, identifying volatile compounds that function as repellants requires further investigation.

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LITERATURE CITED

- Abdollahipour, M., Fathipour, Y., & Mollahosseini, A. (2020). How Does a Predator Find Its Prey? *Nesidiocoris tenuis* is Able to Detect *Tuta absoluta* by HIPVs. *Journal of Asia-Pacific Entomology*, 23(4), 1272–1278. https://doi.org/10.1016/ j.aspen.2020.10.006
- Ammar, E.D., George, J., Sturgeon, K., Stelinski, L.L., & Shatters, R.G. (2020). Asian Citrus Psyllid Adults Inoculate Huanglongbing Bacterium More Efficiently than Nymphs When This Bacterium is Acquired by Early Instar Nymphs. *Scientific Reports*, 10(1), 18244. https://doi.org/ 10.1038/s41598-020-75249-5
- Barman, J.C., Campbell, S.A., & Zeng, X. (2016). Exposure to Guava Affects Citrus Olfactory Cues and Attractiveness to *Diaphorina citri* (Hemiptera: Psyllidae). *Environmental Entomology*, 45(3), 694–699. https://doi.org/10.1093/ee/nvw010
- Cai, Z., Ouyang, F., Su, J., Zhang, X., Liu, C., Xiao, Y., Zhang, J., & Ge, F. (2020). Attraction of Adult *Harmonia axyridis* to Volatiles of the Insectary Plant *Cnidium monnieri*. *Biological Control*, 143,

104189. https://doi.org/10.1016/j.biocontrol. 2020.104189

- Chen, L., Liu, Y., Wu, F., Zhang, J., Cui, X., Wu, S., Deng, X., & Xu, M. (2023). *Citrus tristeza virus* Promotes the Acquisition and Transmission of '*Candidatus Liberibacter Asiaticus*' by *Diaphorina citri. Viruses*, 15(4), 918. https://doi.org/10.33 90/v15040918
- Conchou, L., Lucas, P., Meslin, C., Proffit, M., Staudt, M., & Renou, M. (2019). Insect Odorscapes: From Plant Volatiles to Natural Olfactory Scenes. *Frontiers in Physiology*, 10, 972. https://doi.org/10.3389/fphys.2019.00972
- Das, P., Morawo, T., & Fadamiro, H. (2017). Plant-Associated Odor Perception and Processing in Two Parasitoid Species with Different Degrees of Host Specificity: Implications for Host Location Strategies. *Journal of Insect Physiology*, 101, 169–177. https://doi.org/10.1016/j.jinsphys. 2017.08.002
- David, E., & Niculescu, V.C. (2021). Volatile Organic Compounds (VOCs) as Environmental Pollutants: Occurrence and Mitigation Using Nanomaterials. *International Journal of Environmental Research Public Health*, 18(24), 13147. https://doi.org/10.3390/ijerph182413147
- George, J., Robbins, P.S., Alessandro, R.T., Stelinski, L.L., & Lapointe, S.L. (2016). Formic and Acetic Acids in Degradation Products of Plant Volatiles Elicit Olfactory and Behavioral Responses from an Insect Vector. *Chemical Senses*, 41(4), 325– 338. https://doi.org/10.1093/chemse/bjw005
- Glinwood, R., Ahmed, E., Qvarfordt, E., Ninkovic, V., & Pettersson, J. (2009). Airborne Interactions between Undamaged Plants of Different Cultivars Affect Insect Herbivores and Natural Enemies. *Arthropod-Plant Interactions*, 3(4), 215–224. https://doi.org/10.1007/s11829-009-9072-9
- Gómez-Marco, F., Gebiola, M., Simmons, G.S., & Stouthamer, R. (2022). Native, Naturalized and Commercial Predators Evaluated for Use against *Diaphorina citri*. Crop Protection, 155, 105907. https://doi.org/10.1016/j.cropro.2022. 105907

- Gottwald, T. R., Hall, D. G., Kriss, A. B., Salinas, E. J., Parker, P. E., Beattie, G. A. C., & Nguyen, M. C. (2014). Orchard and Nursery Dynamics of the Effect of Interplanting Citrus with Guava for Huanglongbing, Vector, and Disease Management. *Crop Protection*, 64, 93–103. https://doi.org/10.1016/j.cropro.2014.06.009
- Huang, D., Sun, M., Han, M., Zhang, Z., Miao, Y., Zhang, J., & Yao, Y. (2020). Volatile Organic Compounds (VOCs) Regulate the Spatial Distribution of Lepidoptera Insects in an Orchard Ecosystem. *Biological Control*, 149, 104311. https://doi.org/10.1016/j.biocontrol.2020.104311
- Hung, T., Hung, S., Chen, C., Hsu, M., & Su, H. (2004). Detection by PCR of Candidatus Liberibacter asiaticus, the Bacterium Causing Citrus Huanglongbing in Vector Psyllids: Application to the Study of Vector–Pathogen Relationships. *Plant Pathology*, 53, 96–102. https:// doi.org/10.1046/j.1365-3059.2003.00948.x
- Irvin, N. A., Pierce, C., & Hoddle, M. S. (2021). Evaluating the Potential of Flowering Plants for Enhancing Predatory Hoverflies (Syrphidae) for Biological Control of *Diaphorina citri* (Liviidae) in California. *Biological Control*, 157, 104574. https://doi.org/10.1016/j.biocontrol.2021.104574
- Killiny, N., Nehela, Y., George, J., Rashidi, M., Stelinski, L.L., & Lapointe, S.L. (2021). Phytoene Desaturase-Silenced Citrus as a Trap Crop with Multiple Cues to Attract *Diaphorina citri*, the Vector of Huanglongbing. *Plant Science*, 308, 110930. https://doi.org/10.1016/j.plantsci.20 21.110930
- Lee, S., Km, Y. S., Choi, H. K., & Cho, S. K. (2011). Determination of the Volatile Components in the Fruits and Leaves of Guava Plants (*Psidium* guajava L.) Grown on Jeju Island, South Korea. *Journal of Essential Oil Research*, 23(6), 52–56.
- Li, S., Yuan, X., Xu, Y., Li, Z., Feng, Z., Yue, X., & Paoletti, E. (2021). Biogenic Volatile Organic Compound Emissions from Leaves and Fruits of Apple and Peach Trees during Fruit Development. *Journal of Environmental Sciences*, 108,

152–163. https://doi.org/10.1016/j.jes.2021. 02.013

- Lin, T., Zhu, G., He, W., Xie, J., Li, S., Han, S., Li, S., Yang, C., Liu, Y., & Zhu, T. (2022). Soil Cadmium Stress Reduced Host Plant Odor Selection and Oviposition Preference of Leaf Herbivores through the Changes in Leaf Volatile Emissions. *Science of The Total Environment*, 814, 152728. https://doi.org/10.1016/j.scitotenv.20 21.152728
- Monzo, C., & Stansly, P.A. (2017). Economic Injury Levels for Asian Citrus Psyllid Control in Process Oranges from Mature Trees with High Incidence of Huanglongbing. *PLoS ONE*, *12*(4), e0175333. https://doi.org/10.1371/journal. pone.0175333
- Mozaffar, A., Schoon, N., Bachy, A., Digrado, A., Heinesch, B., Aubinet, M., Fauconnier, M.-L., Delaplace, P., du Jardin, P., & Amelynck, C. (2018). Biogenic Volatile Organic Compound Emissions from Senescent Maize Leaves and a Comparison with Other Leaf Developmental Stages. *Atmospheric Environment*, 176, 71–81. h t t p s : / / d o i . o r g / 10.1016 / j.atmosenv.2017.12.020
- Nakashima, K., Ohitsu, Y., & Prommintara, M. (1998). Detection of Citrus Organism in Citrus Plants and Psylla *Diaphorina citri* in Thailand. *Annals of the Phytopathological Society of Japan*, 64, 153–159.
- Ninkovic, V., & Åhman, I. M. (2009). Aphid Acceptance of Hordeum Genotypes is Affected by Plant Volatile Exposure and is Correlated with Aphid Growth. *Euphytica*, 169(2), 177. https://doi.org/10.1007/s10681-009-9918-3
- Nurhadi. (2015). Huanglongbing Disease (*Candidatus Liberibacter asiaticus*) of Citrus Plants: Threats and Control Strategies. *Pengembangan Inovasi Pertanian*, 8(1), 21–32.
- Nusra, M.S.F., Udukala, D.N., Amarasinghe, L.D., & Paranagama, P.A. (2021). Volatiles from host plant brinjal attract the brinjal Fruit and Shoot Borer -Leucinodes orbonalis Guenee. *Journal of Asia-Pacific Entomology*, 24(3), 695–703. https:// doi.org/10.1016/j.aspen.2021.06.002

- Onagbola, E.O., Rouseff, R.L., Smoot, J.M., & Stelinski, L.L. (2011). Guava Leaf Volatiles and Dimethyl Disulphide Inhibit Response of *Di-aphorina citri* Kuwayama to Host Plant Volatiles: Effect of Guava Leaf Volatiles on *D. citri. Journal of Applied Entomology*, 135(6), 404–414. https://doi.org/10.1111/j.1439-0418.2010.01565.x
- Pichersky, E., & Gershenzon, J. (2002). The Formation and Function of Plant Volatiles: Perfumes for Pollinator Attraction and Defense. *Current Opinion on Plant Biology*, 5(3), 237–243. https://doi.org/10.1016/s1369-5266(02)00251-0
- Poerwanto, M. E., & Solichah, C. (2020). The Repellency of Guava Shoots Extract to The Asian Citrus Psyllid (*Diaphorina citri*). Proceeding International Conference on Green Agro-Industry, 4, 209–214.
- Poerwanto, M.E., Trisyono, Y.A., Subandiyah, S., Martono, E., Holford, P., & Beattie, G.A. C. (2008). Effects of Mineral Oils on Host Selection Behavior of Diaphorina citri. *Jurnal Perlindungan Tanaman Indonesia*, 14(1), 23–28.
- Poerwanto, M., Trisyono, Y., Martono, E., Beattie, G., Holford, P., & Subandiyah, S. (2012). Olfactory Responses of the Asiatic Citrus Psyllid (*Diaphorina citri*) to Mineral Oil-Treated Mandarin Leaves. *American Journal of Agricultural* and Biological Sciences, 7(1), 50–55. https://doi. org/10.3844/ajabssp.2012.50.55
- Rahman, V.J., & Babu, A. (2021). Herbivore-Induced Plant Volatiles from Red Spider Mite, Oligonychus coffeae Infested Tea Plants as Attractant Cues for the Predatory Mite, *Neoseiulus longispinosus. Materials Today: Proceedings*, 41, 613–617. https://doi.org/10.1016/j.matpr.20 20.05.259
- Ramadhan, T.H., Trisyono, Y.A., Mahrub, E., Wijonarko, A., Subandiyah, S., & Beattie, G.A.C. (2008). Pengaruh Jenis Mangsa dan Suhu pada Perkembangan *Menochilus sexmaculatus* Fabricius (Coleoptera: Coccinellidae) dan Peranannya dalam Pengendalian *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Jurnal Perlindungan Tanaman Indonesia*, 14(1), 29–34.

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- Rouseff, R.L., Onagbola, E.O., Smoot, J.M., & Stelinski, L.L. (2008). Sulfur Volatiles in Guava (*Psidium guajava* L.) Leaves: Possible Defense Mechanism. Journal of Agricultural and Food Chemistry, 56(19), 8905–8910. https://doi.org/ 10.1021/jf801735v
- Rugno, G.R., Cuervo, J.G.B., Garcia, A.G., Qureshi, J., & Yamamoto, P.T. (2021). Abundance and Diversity of Lacewings in Grower Operated Organic and Conventional Pest Management Programs for *Diaphorina citri* (Hemiptera: Liviidae). *Crop Protection*, 146, 105682. https://doi. org/10.1016/j.cropro.2021.105682
- Sagrero-Nieves, L., Bartley, J.P., & Provis-Schwede, A. (1994). Supercritical Fluid Extraction of the Volatile Components from the Leaves of *Psidium guajava* L. (Guava). *Flavour and Fragrance Journal*, 9(3), 135–137.
- Seo, M., Rivera, M.J., Stelinski, L.L., & Martini, X. (2018). Ladybird Beetle Trails Reduce Host Acceptance by *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). *Biological Control*, 121, 30–35. https://doi.org/10.1016/j.biocontrol.20 18.02.005
- Silva, J.A.A., Hall, D.G., Gottwald, T.R., Andrade, M.S., Maldonado, W., Alessandro, R.T., Lapointe, S.L., Andrade, E.C., & Machado, M.A. (2016). Repellency of Selected *Psidium guajava* Cultivars to the Asian Citrus Psyllid, *Diaphorina citri. Crop Protection*, 84, 14–20. https://doi.org/10.1016/ j.cropro.2016.02.006
- Sørensen, M., Rinnan, R., Woodrow, I., Møller, B.L., & Neilson, E.H.J. (2020). The Entangled Dynamics of Eucalypt Leaf and Flower Volatile Emissions. *Environmental and Experimental Botany*, 176, 104032. https://doi.org/10.1016/ j.envexpbot.2020.104032
- Supriyanto, A., & Whittle, A. (1991). Citrus Rehabilitation in Indonesia. Proceeding of Eleventh IOCV Conference, 409–413.
- Teixeira, D., Ayers, J., Danet, L., Jagoueix-Eveillard, S., Saillard, C., & Bové, J. (2005). First Report of a Huanglongbing-like Disease of Citrus in São Paulo State, Brazil, and Association of a New Liberibacter Species, 'Candidatus Liberibacter

americanus', with the Disease. *Plant Disease*, *89*(1), 107–107. https://doi.org/10.1094/pd-89-0107a

- Xu, C., Liang, Z., Tang, D., Xiao, T., Tsunoda, M., Zhang, Y., Zhao L., Deng S., & Song, Y. (2017).
 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Volatile Components from Guava Leaves. *Journal of Essential Oil Bearing Plants*, 20(6), 1536–1546.
- Zaka, S.M., Zeng, X.-N., Holford, P., & Beattie, G.A.C. (2010). Repellent Effect of Guava Leaf Volatiles on Settlement of Adults of Citrus Psylla, *Diaphorina citri* Kuwayama, on Citrus. *Insect Science*, 17(1), 39–45. https://doi.org/ 10.1111/j.1744-7917.2009.01271.x