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The response of *Diaphorina citri* to Various Guava Shoots

Mofit Eko Poerwanto, Chimayatus Solichah, Adi Ilcham

Universitas Pembangunan Nasional Veteran Yogyakarta
E-mail address mofit.eko@upnyk.ac.id; E-mail address chimayatus@gmail.com;

Abstract

Citrus production in Indonesia and a part of the world facing greening or CVPD disease is the disease that has the most devastating impact. Diaphorina citri plays an important role as a pathogen main vector. Guava leave raise as a potential control means to reduce D. citri population. The investigation was conducted to determine the response of D. citri to various guava shoots. Responses to ground dried upper shoots of 50oC and 80oC of white, non-seed, and red guava shoots to ten mixed genders of adult psyllids were investigated in a Y-tube olfactometer. As the result, D. citri adults refused to move into guava shoots odors sources. The number of D. citri moved to extract the source of upper of non-seed and red guava shoots extract is 3.80±0.31 and 2.53±0.22 respectively. D. citri population move is 5.47±0.22 and 2.60±0.13 for white guava and red guava odor sources respectively. The responses decreased as the increase in drying temperature. The lowest response was found from red guava, followed by non-seed guava in the middle level, and white guava shoots as the highest. It is suggested that shoots properties of red guava have the highest potency for reducing the D. citri population. However, properties of red guava upper shoot still have an attractive effect on M. sexmaculatus, a predator of D. citri.

Keywords: Diaphorina citri, M. sexmaculatus, guava, CVPD, olfactometer

I. INTRODUCTION

Citrus (*Citrus reticulate*), in Indonesia mostly produced from East Java and North Sumatra. Production growth was unstable due to the pathogen of Citrus vein phloem degeneration (CVPD), however, it is predicted that citrus production is 3,250,000 tons in 2020, with the increase per year on average is about 4.93%. Household consumption was 3.41 kg/cap/year (882,689 tons) in 2016 and the demand was projected to increase 3.73% up to 2020 (Ministry of Agriculture, 2015). Reduction of *D. citri* population and the incidence of CVPD disease was found in citrus plantations on the presence of guava plants (Zaka *et al.,* 2011), (Pustika *et al.,* 2008), but it has not been investigated yet about the variation ability of repellent among the varieties of guava plants.

II. LITERATURE REVIEW

Citrus vein phloem degeneration (CVPD), also recognize as Greening or Huanglongbing disease is a very important problem in the citrus plantation that has a high impact on citrus production. The disease up to know impacting on shortens the productive life, decreases the citrus fruit production and quality, high mortality rate, which the impact will weaken the fulfill and competitiveness the needs of citrus fruit product (Nurhadi, 2015). Consequently, a significant decrease in citrus production appears in Indonesian from 2008 (2,467,632 ton) to 2012 (1,611,768 ton), and it will turn the increase of citrus fruits imports up to 256,000 ton in 2012 that valued at US$ 247,000,000, from 138,000 ton in 2008 with a value of US$117,000,000 (Ministry of Agriculture, 2015).

In Asia and Africa, *Candidatus Liberibacter asiaticus* and *Candidatus Liberibacter africanus* are the pathogen causes of CVPD, and in America, CVPD causes by *Candidatus Liberibacter americanus*. It is a gram-negative bacteria (Nakashima *et al.,* 1998), (Teixeira *et al.,* 2005). The disease only transmitting by the 4-5 instar nymph and the imago of *Diaphorina citri* (Capoor *et al.,* 1774), (Xu *et al.,* 1988). The capability lasts a lifetime (Xu *et al.,* 1991), (Hung *et al.,* 2004). The four major components are implemented for controlling CVPD (Supriyanto & Whittle, 1991), consisted of (1) quarantine, (2) disease-free seeds planting, (3) *D. citri* control, and (4) elimination of infected plants. Synthetic insecticides still the main means for controlling the CVPD disease vector (*D. citri*) with a high enough cost (Monzo & Stansly, 2017). The use of mineral oil is quite effective and harmless to the environment (Poerwanto *et al.,* 2012), but then it is expensive and in low availability in Indonesia.

III. RESEARCH METHODOLOGY

Methodology explains what research method is used, how the data collected and proceeds quantitatively or qualitatively to get more explanation in the result and discussion.

III.1. D. citri cultures and M. sexmaculatus

Diseases-free culture of *D. citri* adults was supplied from Citrus and Subtropical Plants Research Station. They were cultured on *Murraya paniculata* L. inside cages covered by nylon mesh (1000 mm high, 600 mm long, 600 mm wide) inside a greenhouse with 26 ± 4°C and 60 - 80% RH. *M. Sexmaculatus* beetles were obtained from the area of long beans plantation. They were the predator of aphids that sucks plant shoots.

III.2. Leaf extract

The first and second fully open leave from the top of guava leave and citrus (*Citrus reticulate*) were collected. They were dried at a temperature of 50^0C for 24 hours and a temperature of 80^0C for 48 hours in an electrical oven. The leave that has been dried were powdered by grinding with an electric grinder and sieved to avoid unwanted granules from the powder. The airtight containers were used to store leave powder before application.

III.3. Olfactometer responses

Construction of Y-tube olfactometer was from a 300 mm long, transparent glass tube, 10.0 mm diameter (internal). Silicone tube (5.0 mm internal diameter) was connected to a sucker to suck air which the flow of 10 mL minute^{-1}into the olfactometer, as measured with a flow meter. Silicone tube also conected each arm of Y-tube to each of the odour sources. Activated charcoal and distilled water container were installed for filtering and humidifying air entering each olfactometer by passing it through before entering a plastic container (50 mm diameter × 40 mm high) contained odor source. Dried leave extract of 25 mg for each odour source. Responses of mixed gender adult of *D. citri* were investigated for the comparisons treatment as followed in Table 1. plastic tubes (31.5 mm diameter and 50 mm long) were used to collect adult of *D. citri*. They have released through the distal end of the olfactometer that was used for tests after they were starved for 60 minutes. The activity of adults *D. citri* was recorded within this olfactometer for 30 minutes, then removed for 30 minutes, and then released into another olfactometer for 30 minutes for the second test. Ten adult *D. citri* were used for each replicate (n = 15). The proportion of adults moved were recorded as responses to the sources of the volatile odor. The same procedure has been conducted to *M. sexmaculatus* adults towards citrus and fresh air odors source.

Table 1. Comparisons in Y-tube olfactometer: ten adult *D. citri* (I – VII) and ten *M. sexmaculatus* adults (VIII) in each comparison per replicate (n = 15) to odor from sources of leave extracts (1:1 w/w) dried at 50^{0}C for 24 h (50) and dried at 80^{0}C for 48 h (80) listed in the left hand and right-hand column were recorded in intervals of 30 minute

Compariso n	Sources of upper shoot odor for *D.citri*		
I	Fresh air	Vs	Citrus (50)
II	Red guava (50) + Citrus	Vs	No need for guava (50) + Citrus
III	Red guava (50) + Citrus	Vs	White guava (50) + Citrus
IV	No need for guava (50) + Citrus	Vs	White guava (50) + Citrus
V	Red guava (50) + Citrus	Vs	Red guava (80) + Citrus
VI	No need for guava (50) + Citrus	Vs	No need for guava (80) + Citrus
VII	White guava (50) + Citrus	Vs	White guava (80) +Citrus
	Sources of upper shoot odor for *M. sexmaculatus*		
VIII	Red guava (50)	Vs	Citrus (50)

IV. FINDING AND DISCUSSION

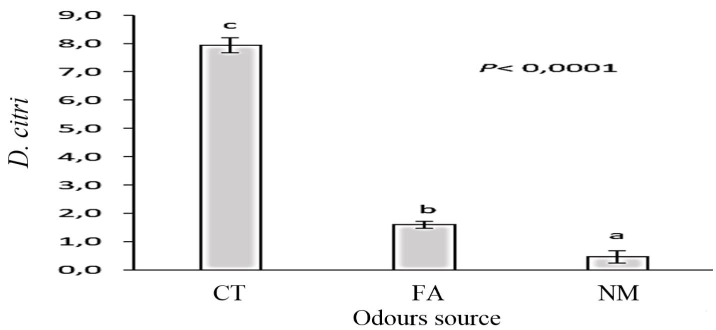

Figure 1. Average (± Standard error) of *D. citri* adults moved or not moved (NM) toward volatile odor of citrus leaves or fresh air entering Y-tube olfactometers

The response of *D. citri* and *M. sexmaculatus* adults to the odor of plant leaf extracts was effectively tested by using a Y-tube olfactometer. Observation results in comparison I showed that the number of *D. citri* adults moved towards the source of the odor of citrus leaves was 7.83 ± 0.25, significantly higher than the source without odor or fresh air (1.80 ± 0.13) (Figure 1.).

Response's variation of *D. citri* adult to the guava leaves extract mixed with citrus leave odor sources (1:1 w/w) was investigated in every set test of Y-tube olfactory. Figures 1 to 3 show the results of the olfactometer experiments. Some of *the D. citri* adults seen did not move to both of odour sources. It is proved that guava leave odor source has a repellent effect that prevents *D. citri* adults from choosing the odor of citrus leave or their host plant. The same response of *D. citri* to guava leave extract when covered by spraying on citrus leave also found by Barman and Zeng (2014), and Barman *et al.,* (2016). The existence of guava leave odor in the citrus plantation was observed by Onagbola *et al.,* (2011) and Poerwanto *et al.,* (2008), could reduce *D. citri* adult population in citrus leaves. Significantly less *D. citri* adult move to the treatment of citrus dried leave with mix with dried leave of upper shoots of red guava than upper shoots of non-seed guava which have been dried at a temperature of 50^{0}C for 24 hours (P< 0.0001). *D. citri* adult move to extract of citrus leave mixed with non-seed guava upper shoot and red guava upper shoots leave extract is 3.80±0.31 and 2.53±0.22 respectively. The number of *D. citri* adult not moved are the same with the number of the *D. citri* adult move to the odor source of upper shoots of non-seed guava.

White guava dried upper shoot with the addition of dried leave of citrus is also more attracts *D. citri* adult compared to dried upper shoot red guava with the addition of citrus dried leave mixture of (P< 0.0001). The number of *D. citri* move to white guava odor source is 5.47±0.22 and less *D. citri* move to red guava odor sources (2.60±0.13).

D. citri adult attracted to the non-seed guava dried upper shoot in the addition of citrus dried leave was lower than to white guava with the addition of citrus dried leave in a paired comparison test. Non-seed guava was significantly higher (P< 0.0001) in repelling *D. citri* than white guava. The number of *D. citri* moved was on average 2.63±0.32 for non-seed guava dried upper shoot odor source and 4.80±0.23 for white guava.

Responses of *D. citri* adult that appeared to the guava dried shoots drying at a temperature of 50^{0}C for 24 hours and a temperature of 80^{0}C for 48 hours (Figure 1, 2, and 3) showed that upper shoot (fully opened leave number one and two from the bud) that dried at 80^{0}C for 48 hours has higher attractants effect (P< 0.0001) to *D. citri* adult than shoot dried at 50^{0}C for 24 hours. *D. citri* adult move to upper shoot dried at 80^{0}C for 48 hours and at 50^{0}C for 24 hours was 2.07±0.18 and 3.80±0.31, and 5.47± 0.22 and 2.80± 0.11, and 1.33±0.16 and 2.53±0.22, for white guava, non-seed, and red respectively. It also suggested that the dose of guava leave properties controls the repellency effect (Barman & Zeng, 2014) and it is suggested that upper shoots of guava have a higher dose based on the higher effect found on it. The attractiveness of *D. citri* adults is also controlled significantly by drying temperature and drying duration of guava shoots.

Based on the comparison of the dried upper shoot of white guava, non-seed, and red, it is suggested that the highest repellent effect to *D. citri* adult has found to the odors of red guava, and non-seed guava at the second level, and the less effect of repellency found in white guava. Volatile compounds are used by *D. citri* adults to locate the position of their host plants. It is also used for finding plants' parts that still lack other insect pests. The cue is specific species and intensities (Zaka *et al.,* 2011), (Poerwanto, 2013).

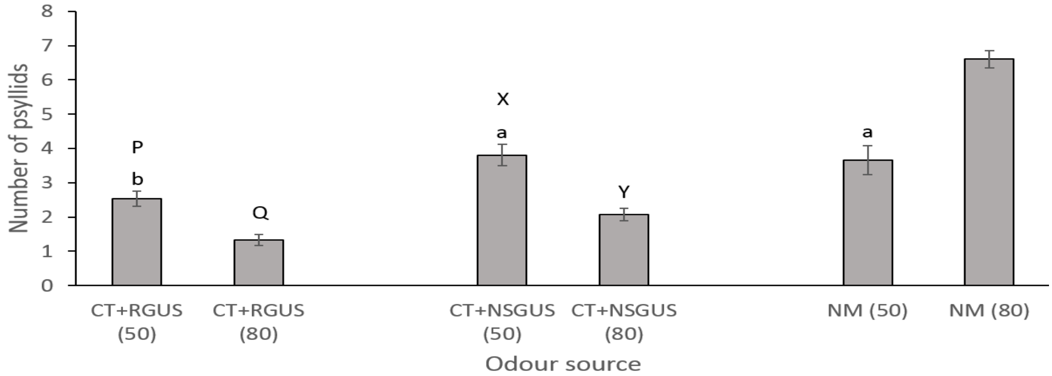

Figure 2. Average (±Standard error) of *D. citri* adults moved towards volatiles or not moved (NM) to enter Y-tube olfactometers: response to a mixture of upper shoots of red guava + citrus (CT + RGUS) and upper shoots of non-seed guava + citrus (CT + NSGMS) leave extract dried at 80^{0}C for 48 hours (80) and 50^{0}C for 24 hours (50).

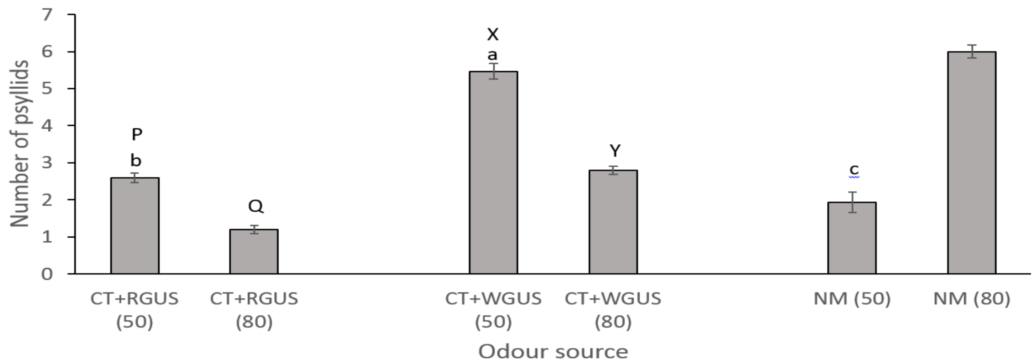

Figure 3. Average (±Standard error) of *D. citri* adults that moved towards volatiles or not moved (NM) to enter Y-tube olfactometers: response to mixture leave extract of upper shoots of red guava + citrus (CT + RGUS) and upper shoots of white guava + citrus (CT + WGMS) leave extract dried at 80^{0}C for 48 hours (80) and 50^{0}C for 24 hours (50).

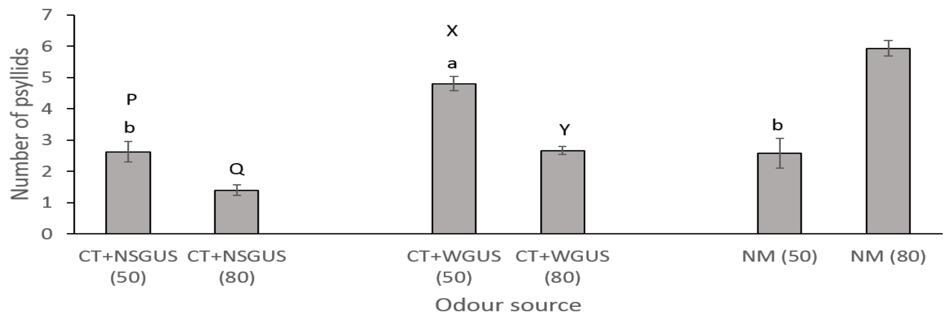

Figure 4. Average (±Standard error) of *D. citri* adults that moved towards volatiles or not moved (NM) entering Y-tube olfactometers: response to mixture leave extract of upper shoots of non-seed guava + citrus (CT + NSGUS) and upper shoots of white guava + citrus (CT + WGMS) leave extract dried at 80^{0}C for 48 hours (80) and 50^{0}C for 24 hours (50).

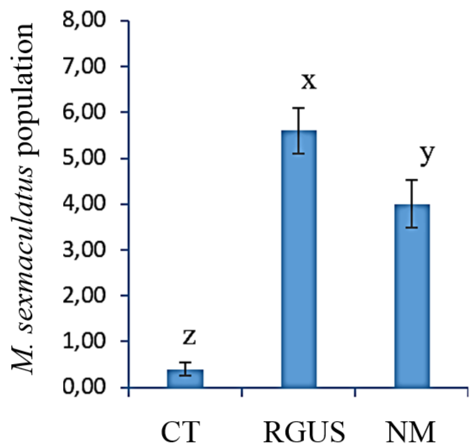

Figure 5. Average (±SE) *M. sexmaculatus* adults not moved (NM), moved to citrus leave (CT), and red guava upper shoot (RGUS) in Y-tube olfactometer

The *M. sexmaculatus* adults is a polyphagous insect predator with many kinds of prey, especially bugs. This beetle uses the odors (volatile) of its host insect prey to find its prey. In comparison VIII (Figure 5.), it can be seen that red guava shoot leaves are significantly ($P <0.0001$) more attractive than citrus shoot leaves with populations of 5.60 ± 0.49 and 0.60 ± 0.21, respectively. It is suggested that the content of secondary metabolic substances which are repellent for *D. citri* adults do not have any effect to attract *M. sexmaculatus*. Besides that, there was also the mixing of the odors of the two types of leaves which resulted in some of the *M. sexmaculatus* population (4.19 ± 0.14) immobile (NM) to one of the sources of the odor. Herbivorous insects use volatile compounds with specific types and intensities as sources of chemical markers to recognize the position of their host plants (Campbell & Borden, 2006), and find of plant's parts that are still free from other insects pests which are competitors (Pallini *et al.*, 1997). Likewise, predatory insects also use the same compounds to find plants or insect habitat for prey. These volatile compounds will be captured by the receptor neurons on the antenna. The response depends on the type and intensity of the compound being caught. The response will be higher with the increasing intensity of the captured volatile compounds (Bichao *et al.*, 2005). In finding the host, the insect *D. citri* and its predator *M. sexmaculatus* also rely on the presence of specific volatile compounds from citrus shoots. Formic compounds and acetic acids play a role in host selection (George *et al.*, 2016).

V. CONCLUSION AND FURTHER RESEARCH

Repellent effect to *D. citri* adult is found in guava leave properties. The repellent effect reached in highest level was red guava leave, and non-seed guava as the second level, and less repellent effect in white guava. The drying methods determined the repellent effect. The same phenomenon was not found in *M. Sexmaculatus*, the predator of *D. citri.*

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