Repelence Effect

by Mofit Eko Poerwanto

Repellence Effect of Various Parts of Guavas Shoot to Asian Citrus Psyllid (*Diaphorina citri* Kuwayama)

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***Abstract*—CVPD or Huanglongbing is the most devastating disease on citrus production in Indonesia and in the world. It is vectored by asian citrus psyllid. Guava leave extract is a prospective control means for reducing psyllid population. Research was conducted to investigate the repellent effect of guava shoots to psyllids. Repellent effect of grinded dried upper shoot (leaf number 1 and 2 from the top), middle shoot (leaf number 3 and 4 from the top), and bottom shoot (leaf number 5 and 6 from the top) of red, white, and non-seed guava leave to ten adult psyllids of mixed gender were determined in Y-tube olfactometer. The result shows that guava leave has repellence effect to psyllids adult. The effect is reduced as the increase of leaf age. Highest repellence effect is found from red guava leave, followed by non-seed guava and white guava. It is suggested that the highest repellent properties is in upper shoots or youngest leaf of guava. Olfactometer: a device used to study insect behavior in presence of an olfactory stimulus. It consists of a tube with a bifurcation ("Y" shape) where an insect walks and decides between two choices, usually clean air versus air carrying an odor.**

***Index Terms*—Diaphorina citri, guava, huanglongbing, leave volatile.**

I. Introduction

Mandarin (*Citrus reticulata*) and pomelo (*Citrus maxima* or *C. grandis*) are widely grown citrus varieties in Indonesia. They are located in some production centers. North Sumatra and East Java are as the center of mandarin production in Indonesia. While the centers of pomelo are in South Sulawesi and East Java. Production growth in 2011-2015 is decline in locations of outside Java but showed an increase in Java. Production of mandarin in Indonesia mostly comes from North Sumatra and East Java. Citrus production in Indonesia in 2020 is estimated 3.25 million tons, with the average increase 4.93% per year. Citrus consumption by household in 2016 was 3.41 kg/cap/year or 882,689 tons for Indonesian population. The demand of citrus fruit for households are projected to increase over the next five years (2016-2020) with an average of 3.73% [1].

The most serious problem in citrus production is the widespread of Citrus vein phloem degeneration (CVPD) or Huanglongbing or Greening disease. Besides impacting on the high mortality rate, CVPD also shortens the productive life of the citrus plants as well as decreases the productivity and product quality which in turn will weaken the competitiveness and fulfill the product needs [2]. The impact of CVPD is marked by a decrease in Indonesian citrus production from 2,467,632 tons in 2008 to 1,611,768 tons in 2012, and increased imports of citrus fruits from 138,000 tons with a value of USD117 million in 2008 to 256,000 tons with a value of USD247 million in 2012 [1].

The pathogen causes of CVPD are Gram-negative bacteria '*Candidatus Liberibacter asiaticus*' and '*Candidatus Liberibacter africanus*' for Asian and African types and '*Candidatus Liberibacter americanus*' for American type [3], [4]. Only the imago psylids and the 4-5 instar nymph are capable of transmitting the disease [5], [6]. Insect vector is able to transmit disease throughout its life [7], [8]. The control of CVPD is implemented through application of four major components [9], namely (1) the use of disease-free seeds, (2) elimination of infected plants in the field, (3) vector insect control, and (4) quarantine. Control of CVPD disease vector insects (*D. citri*) still focuses on the use of synthetic insecticides that require a high enough cost to keep infected plants in production [10]. Alternative substitute for synthetic insecticides is the use of mineral oil [11], but the availability is still low and it is expensive. The presence of guava plants in citrus plantations was able to reduce the population of psylids and the incidence of CVPD disease [12], [13], but the types and part of guava plants which has capability of reducing the psyllids population are not known.

II. Materials and Methods

A. Psyllid Cultures

Disease-free culture of psyllids were obtained from Citrus and Subtropical Plants Research Station and were maintained on the ornamental orange jasmine plants (*Murraya paniculata* L. [Sapindales: Rutaceae: Aurantioideae: Aurantieae] in nylon mesh cages (600 mm long, 600 mm wide, 1000 mm high) in a greenhouse at 26±4°C and 60-80% relative humidity.

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TABLE I. Y-TUBE OLFACTOMETER COMPARISONS: FOR EACH COMPARISON, RESPONSES OF 10 ADULT *D. CITRI* PER REPLICATE (N = 30) TO AROMA FROM SOURCES OF LEAVE EXTRACTS (1:1 W/W) LISTED IN THE LEFT HAND AND RIGHT HAND COLUMN WERE RECORDED OVER 30 MIN INTERVALS

Comparison	Aroma sources		
I	Citrus shoot	Vs	Filtered air
II	Citrus + Red guava bottom shoot	Vs	Citrus + Red guava upper shoot
III	Citrus + Red guava middle shoot	Vs	Citrus + Red guava bottom shoot
IV	Citrus + Red guava upper shoot	Vs	Citrus + Red guava middle shoot
V	Citrus + Non seed guava bottom shoot	Vs	Citrus + Non seed guava upper shoot
VI	Citrus + Non seed guava middle shoot	Vs	Citrus + Non seed guava bottom shoot
VII	Citrus + Non seed guava upper shoot	Vs	Citrus + Non seed guava middle shoot
VIII	Citrus + White guava bottom shoot	Vs	Citrus + White guava upper shoot
IX	Citrus + White guava middle shoot	Vs	Citrus + White guava bottom shoot
X	Citrus + White guava upper shoot	Vs	Citrus + White guava middle shoot
XI	Citrus + Red guava bottom shoot	Vs	Citrus + Non seed guava bottom shoot
XII	Citrus + Red guava bottom shoot	Vs	Citrus + White guava bottom shoot
XIII	Citrus + Non seed guava bottom shoot	Vs	Citrus + White guava bottom shoot

B. *Leaf Extract*

Citrus (*Citrus reticulata*) shoots with 2 (two) fully open leave, and guava leave from the upper shoots (leaf number 1-2 from the top), middle shoots (leaf number 3-4 from the top), and bottom shoots (leaf number 5-6 from the top) were collected and then dried in oven at 50^{0}C temperature for 24 h. The dried leave were ground to powder with an electric grinder and then sieved to avoid unwanted granules from the powder. The leave powder was stored in airtight containers.

C. *Olfactometer Responses*

Y-tube olfactometer was constructed from a 10.0 mm diameter (internal), 300 mm long, transparent glass tube, connected by a 5.0 mm diameter (internal) silicone tube to a sucking machine that was used to suck air into the olfactometer at 141 mL min^{-1}, as measured with a flow meter (Model N 112-02G, Cole-Parmer Instrument Company, Illinois, USA). Each arm of Y-tube was connected to one of the two aroma sources with silicone tubing. Air entering each olfactometer was filtered through activated charcoal and humidified by passing it through distilled water before it was passed through a transparent plastic container (50 mm diameter × 40 mm high) housing the aroma source (treatment). Each aroma source was 25 mg dried leave extract. Responses of adult psyllids of mixed gender were determined for the paired treatment comparisons listed in Table 1. Adult psyllids were collected in specimen tubes (31.5 mm internal diameter and 50 mm long). The specimen tubes were open at one end and covered by fine mesh at the other. The open end was immediately sealed with rubber plug after the psyllids were collected. The psyllids were then starved for 60 min before they were released into the distal end of the olfactometer used for the first of two tests (Table 1).

The adults were left within this olfactometer for 30 min, then removed, kept within a covered specimen tube for 30 min and then released into a second olfactometer for 30 min for the second test. Ten adult psyllids were used for each replicate (n = 30 for each paired treatment). Responses to the volatile aroma sources were recorded as the proportion of adults observed during a 30 min period moving beyond the distal end of the olfactometer. All observations were undertaken in laboratory at 27-29°C. Both olfactometers were washed with soapy water and then rinsed in tap water and 90% ethanol between replicates.

D. *Statistical Analysis*

Differences between treatments were determined using SPSS (Version 21). Varian analysis and Duncan test were perform to determine whether the number of psyllids moving or not moving towards either of the two arms of the olfactometer were significantly different.

III. RESULT AND DISCUSSION

The results of the olfactometer experiments are shown in Fig. 1 up to 5. Significantly higher number (8.27 compared to 1.40) of adults observed in comparison (I) moved towards the source of host plant volatiles aroma (citrus leave extract) than towards the source of clean air (Fig 1.). This outcome showed that the olfactometer was an effective means of testing responses of psylid (*D. citri*) adults to plant volatiles aroma.

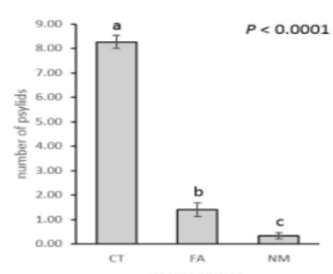

Figure 1. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of Citrus shoots (CT) and to Filtered air without any leave extract (FA).

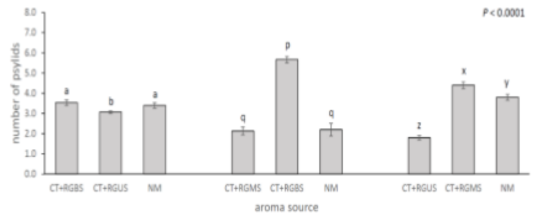

Figure 2. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of citrus + red guava upper shoots (CT + RGUS), of citrus + red guava middle shoots (CT + RGMS), and of citrus + red guava bottom shoots (CT + RGBS).

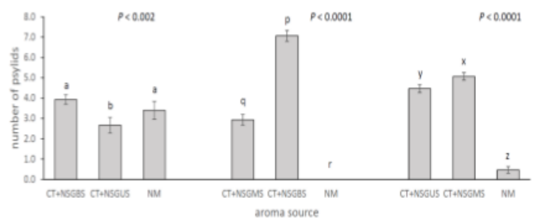

Figure 3. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of citrus + non seed guava upper shoots (CT + NSGUS), of citrus + non seed guava middle shoots (CT + NSGMS), and of citrus + non seed guava bottom shoots (CT + NSGBS).

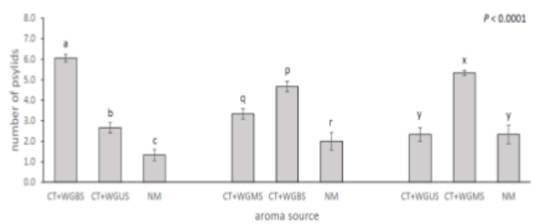

Figure 4. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of citrus + white guava upper shoots (CT + WGUS), of citrus + white guava middle shoots (CT + WGMS), and of citrus + white guava bottom shoots (CT + WGBS)

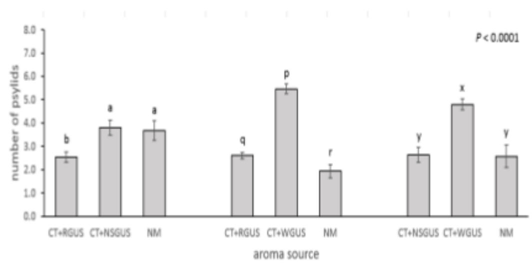

Figure 5. Mean (±SE) of psyllids adults that not moved (NM) or moved towards volatiles entering Y-tube olfactometers: response to leave extract of citrus + red guava upper shoots (CT + RGBS), of citrus + white guava upper shoots (CT + WGBS), and of citrus + non seed guava upper shoots (CT + WGBS).

Variant response of adult psylids to the mixture of plant volatiles aroma sources of citrus leave and guava leave extract (1:1 w/w) is observed in every set of Y-tube olfactory tests. It is also obviously seen that some adult psylids do not move to both of aroma sources. Those phenomenon prove repellence effect of guava leave prevent adult psylids to choose citrus leave extract volatile as cue to their host plant (citrus). The psyllids fail to recognize the aroma of citrus leave. Barman and Zeng [14], [15] also found the same repellence effect of guava leave extract when sprayed on citrus leave. The presence of guava shoot aroma are able to reduce adult psyllids population in citrus leave [16], [17]. More adult psylids move to the citrus dried leave with the addition of bottom shoots of guava dried leave than upper shoots guava dried leave. The number of psylids move to citrus leave extract with the addition of upper shoots leave extract of red guava, non-seed guava, and white guava is 3.07±0.07, 2.67±0.38, and 2.67±0.25 respectively. Those are significantly different to the number of psylids move to citrus leave extract with the addition of bottom shoots leave extract of red guava (3.53±0.13; $P< 0.0001$), non-seed guava (3.93±0.22; $P< 0.002$), and white guava (6.07±0.18; $P< 0.0001$).

Mixture of guava dried bottom shoot and citrus dried leave is more attractive for psylids than mixture of guava dried middle shoot and citrus dried leave. The number of psylids move is 5.67±0.15, 7.07±0.26, and 4.67±0.25 for red guava, non-seed guava, and white guava aroma sources respectively. Whereas the attractiveness of guava dried middle shoot is significantly lower ($P< 0.0001$). It is 2.13±0.19, 2.93±0.26, and 3.33±0.25 psylids move to red guava, non-seed guava, and white guava aroma sources respectively.

Psylids move in paired comparison test to the mixture of guava dried upper shoot and citrus dried leave is lower than mixture of guava dried middle shoot and citrus dried leave. Repellent effect of guava dried upper shoot is significantly higher than guava dried middle shoot ($P< 0.0001$). The number of psylids move is 1.80±0.11, 4.47±0.19, and 2.33±0.33 for red guava, non-seed guava, and white guava upper shoot aroma sources respectively. Most of psylids move to red guava (4.40±0.16), non-seed guava (5.07±0.18), and white guava (5.33±0.13) upper shoot aroma sources, and the rest population is not moving to the both aroma sources.

Based on the responses of psyllids to the part of guava dried shoots (Fig 2, 3, and 4), it can be stated that upper shoot (leave number 1 and 2 from the top) has highest repellence effect to psyllids adult, followed by middle shoot (leave number 3 and 4 from the top), and the lowest effect is observed in bottom shoot (leave number 5 and 6 from the top). The result suggest that the increase of guava leaf age will reduce the repellence effect to psyllids adult. It also suggest that the repellency is dose dependent [14] and highest repellence properties is in upper shoots of guava.

Based on the comparison of red, non-seed, and white guava dried upper shoot, it is suggested that aroma of red guava has highest repellent effect to psyllids adult, followed by non-seed guava, and white guava (Fig 5.). It is suggested that the highest repellent properties is in red guava shoot. Psyllid uses volatile compounds of specific species and intensities as a cue to determine the location of their host plants, and finds parts of plants that are still free of other competing insects [13], [18].

IV. Conclusion

Guava leave has repellent effect to psyllids adult. Highest repellent effect is found from red guava leave, followed by non-seed guava and white guava. The effect is reduced as the increase of leaf age. It is suggested that the highest repellent properties is in upper shoots or youngest leaf of guava.

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Conflict of Interest

The authors declare no conflict of interest

Author Contributions

Mofit Eko Poerwanto: conducted the research experiment, analyzed data, wrote the paper.

Chimayatus Solichah: conducted insects cultures for experiment, leave extraction for experiment.

All authors have approved the final version.

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