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Role of Plant Volatile to *Diaphorina citri* on Feeding and Oviposition Behaviour

Mofit Eko Poerwanto[1], Chimayatus Solichah[2]
[1, 2] Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Yogyakarta, Indonesia

Abstract
Citrus production in Indonesia and in the world is suffered from disease of citrus vein phloem degeneration (CVPD. It was vectored by psylids (Diaphorina citri). The psylids used citrus plant volatiles as cues for finding their host plants for feeding and laying eggs. Extract of guava leaves was a prospective control material for declaining population of psyllid. Investigation by research was conducted to determine the effect of CVPD symptomatic citrus plants in attracting psylids gravid female for staying and laying eggs, and also to determine the repellency ability of guava shoots to adult psyllids. Citrus buds of healthy plants and CVPD symptomatic plants were exposed to ten gravid female and the number of psylids stay and the number of eggs per bud was recorded. Y-tube olfactometer was used to determine repellent effect of upper shoot, middle shoot, and bottom shoot of guava leave to ten adult psyllids. The result shows that CVPD symptomatic plants was more attractive for laying eggs, eventhough the number of eggs was higher on healthy plants. Repellence effect to psyllids adult was identified in guava leave extracts. The increase of leaf age would decrease the effect. It was suggested that repellent properties were highest youngest leaf or upper shoots of guava.

Keywords: CVPD, *D. Citri*, Guava, Repelence, Citrus

INTRODUCTION

According to the Indonesian Central Statistics Agency (BPS) (2020), citrus fruit production of Indonesia in 2015 to 2020 has increased successively, namely 2.13 million tons, 2.29 million tons, 2.51 million tons, 2.56 million tons and 2.72 million tons. Citrus production in 2020 increased by 6.22% or 159.46 thousand tons from 2019 with a total production of 2.72 million tons. The increase was supported by the increase of the number of producing plants. The highest production of citrus in 2020 occurred in the second quarter, which increased by a total of 828.38 thousand tons produced by 19.71 million citur plants from the previous year in 2018 in the second quarter of the total production of 592.93 thousand tons produced by 17.02 million citrus plants.
Citrus is a productive plant but is susceptible to insect pests and diseases that result in a decrease in production. One of devastating pests that attack citrus and cause disease is *Diaphorina citri* Kuw. vector of disease of Citrus Vein Phloem Degeneration (CVPD), also called *Huanglongbing* disease, caused by *Liberibacter asiaticus* (Balitjestro, 2015)

The attack of CVPD has an impact on high mortality, shortens productive life and reduces productivity and quality of citrus fruits. The decrease of Indonesia citrus fruit production was from about 2,467,632 tons (2008) to 1,611,768 tons (2012) and an increase in citrus imports from 138,000 tons with a value of USD117 million in 2008 to 256,000 tons with a value of USD247 million in 2012 (Nurhadi, 2015). Control of *D. citri* or psyllid still relay on the use of synthetic chemical insecticides that are expensive and not environmentally friendly (Monzo & Stansly, 2017). An alternative to synthetic insecticides is mineral oil (Poerwanto *et al.*, 2012), but it is still rare and expensive. Intercroping of guava plants in citrus plantations was significantly decrease the population of psylids and infection of CVPD disease (Pustika *et al.*, 2007; Zaka *et al.*, 2011), but the types and part of guava plants which has capability of reducing the psyllids population are not known. This study was aimed to identify the oviposition and feeding behaviour of *D. citri* and the repellency ability of dried guava leaf extract against *D. citri*.

LITERATURE REVIEW

In the process of searching for host plants, one of the factors that affect insects is volatile compounds released by plants. Volatile compounds are secondary compounds released by plants and evaporate

Corresponding author:
mofit.eko@upnyk.ac.id
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quickly (Sutisna *et al.*, 1988). Each type of plant produce spesific volatile compounds. The content and composition of these compounds have contributed to the distinctive character of the plant. Volatile compounds are produced by plants from the organs of leaves, flowers, or fruit. Volatile compounds secreted by plants can provide differences in the attraction of insects to plants. In addition to volatile compounds, physical and chemical properties can also affect the attractiveness of insects on their host plants.

Volatile compounds act as semiochemicals, as mediators in the interaction of an organism with other organisms, both between plants and insects, between insects and insects and between plants and other animals. Semiochemical compounds are often used as biological control against plant pests. The most common strategy using semiochemicals is to attract, trap, and kill insects.

According to Altieri & Nicholls (2004), insect behaviour in finding host plants are often based on the olfactory mechanism for volatile compounds plant. Insect respond to the odors emitted by plants by visiting the plants. Insect response to odor depends on quality and quantity of stimulus, as well as the condition of insects at the time of stimulation. Besides that the existence of a selected host suitability preference is also one of the the cause of the insect's attraction to its host. The volatile compounds released by the host plant is an effective stimulus for many insects, such as *D. citri* in finding host plants.

Wijaya (2007) stated that the oviposition preference of Diaphorina citri in some citrus and yellow plants showed the average number of eggs laid by female D. citri during their lifetime ranged from 37.33 – 219.67 eggs, with the highest oviposition preference occurring in successive yellow plants. also followed by Siamese Sick, Siamese Healthy, Lime, Lime, Bali, and JC. The results of this study are lower than those reported by Ditlin (1994) with 800 eggs, Trisnawati (1998) with 280-488 eggs, Chen (1998) with 200-800 eggs, Nurhadi *et al*., (1989) with 649.8 ± 103, 5 eggs. The highest *D. citri* oviposition preference in orange jasmin plants was because the plants always had young shoots. This situation is a preferred place for *D. citri* for oviposition. Orange jasmin (*Muraya sp.*) plants are pruning resistant plants and have young buds continuously (Sarwono, 1995). This phenomenon causes D. citri insects to lay their eggs on yellow plants. D. citri females always lay their eggs on the shoots of young plants in groups.

The presence of one guava plant among eight citrus plants succeeded in preventing the entry of *D. citri* populations and the occurrence of CVPD attacks on citrus plants (Pustika et al., 2008; Zaka et al., 2011). The volatile active compounds of guava leaves are a potential source of botanical insecticides, but the type and mechanism of the volatile compounds are unknown. *D. citri* in finding its host also relies on volatile odour of specific compounds from citrus shoots. Acetic acids and formic compounds play an important role in acceptance of host (George, 2016).

CVPD is caused of by a Gram-negative bacteria '*Candidatus Liberibacter asiaticus*' and '*Candidatus Liberibacter africanus*' for Asian and African types and '*Candidatus Liberibacter americanus*' for American type (Nakashima *et al.*1998; Teixeria *et al.*, 2005). Imago psylids and the 4-5 instar nymph are the only stage that have capability of transmitting the disease (Capoor *et al.*, 1974; Xu *et al.*, 1988). The vector can transmit disease throughout its life (Xu *et al.*, 1991; Hung *et al.*, 2004). The application of four major components is done to control of CVPD (Supriyanto, 1991). They are (1) disease-free seeds application, (2) Infected plants elimination, (3) control of insect vector, and (4) quarantine.

RESEARCH METHODOLOGY

D. citri was cultured in a greenhouse on the *Muraya paniculata* plants planted in plastic pots (Ø 25 cm, height 18 cm) in gauze cages (length: 60 cm, width: 60 cm, and height: 100 cm). Eggs for initial rearing was obtained from *M. paniculata* in the field. Rearing room set the temperature at 26-30^{o}C and the relative humidity at 60-70%. Healthy plants and one year old CVPD symptomatic plants were found from a CVPD endemic area in Bayan village, Purworeja Regency, Province of Central Java. Plants are grouped into two groups: healthy plants and plants with CVPD symptoms, based on the presence or absence of CVPD symptoms (leaves are yellow, stiff, standing upright, chlorosis spots, the veins of the leaves are darker green while the leaves is yellow).

Scoring of CVPD symptomatic plants using iodine solution. All CVPD symptomatic plants were tested using an iodine solution according to Lily Eng (2007) method to determine CVPD attack level (scoring). The score used is 0 for healthy plants and 4 for the most severed one. While 1 - 3 were plants with CVPD attack rates between healthy to the worst. Based on the test results (scoring), plants grouped into five groups according to their scores.

Oviposition preference test on citrus with CVPD symptoms was carried out on one year old citrus plants that had four buds with shoot lengths ranging from 6 - 10 mm, on choice trial with 10 replicates. Citrus plants at various CVPD attack levels and healthy ones were arranged randomly in a circle in a gauze cage (length 60 cm, 60 cm wide, and 100 cm high), with a radius of 20 cm from the center of the circle. Thirty gravid female (female containing eggs) of *D. citri* were released from the center circle. Observations on the number of *D. citri* that landed on each test plant were recorded every day at 09.00, 12.00 and 15.00. On the third day each bud was cut and the number of eggs was counted by using a binocular microscope.

Citrus reticulata upper shoots with two fully open leave, and guava 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 dried in oven for 24 hours at 50^0C, then the dried leave were ground with an electric grinder and then sieved to colect the powder. Airtight containers were used to store leave powder.

Repellence test of guava leaf was conducted by using Y-tube olfactometer. It was constructed of transparent glass tube (10 mm internal diameter, 300 mm long), connected by a silicone tube (5 mm internal diameter) to a sucking machine with capacity of air sucking of 20 mL min^{-1}, as measured with an air flow meter. Each arm of Y-tube was connected to one of the two aroma sources with silicone tubing. Air entering each arm of Y-tube olfactometer was filtered by activated charcoal and humidified through distilled water and then it was passed through a transparent glass container (50 mm diameter, 40 mm high) as aplace of the aroma source (treatment). Each aroma source was 25 mg dried leave extract. Paired treatment comparisons listed in Table 1 were used to determine the responses of adult psyllids of mixed gender. Specimen tubes (31.5 mm internal diameter, 50 mm long) were used to collect adult *D. citri* (psyllids). The psyllids were then starved for 60 min before they were released into the distal end of the Y-tube olfactometer (Table 1).

Table 1. Y-tube olfactometer test: each comparison, responses of 10 adult *D. citri* (psylids) per replicate (n = 30) to source of aroma of dried leave extracts (1:1 w/w) listed in the left side and right side column were recorded over 30 min intervals

Comparison	Source of aroma		
I	Citrus (CT)	vs	Citrus + guava upper shoot (CT+GUS)
II	Citrus (CT)	vs	Citrus + guava middle shoot (CT+GMS)
III	Citrus (CT)	vs	Citrus + guava bottom shoot (CT+GBS)

FINDING AND DISCUSSION

Gravid female of *D. citri* prefered to stay for laying eggs on buds of citrus with CVPD symptoms (Table 2.). It was strongly suspected that this was related to the increase of plant-specific volatiles as an effect of disease attack. The number of psylids stayed on healthy citrus buds was significantly lower (2.60 ± 0.27) than on CVPD symptomatic citrus buds (6.85 ± 0.52). The preference of selecting citrus plants with CVPD symptoms was thought to be caused by an increase in specific volatile compounds released by buds (Eigenbrode *et al*., 2002). These compounds are cues of the presence of a host plant that can be used for feeding and laying eggs. It was suspected that citrus leaves attacked by CVPD released more specific volatile compounds than healthy citrus leaves. The compound was used by *D. citri* as a cue in finding its host. The same results were also obtained by Eigenbrode *et al*. (2002) on *Myzus persicae* with potato plants affected by potato leafroll virus disease. The volatile compounds produced by virus infected potato plants were higher than that of healthy plants. The proportion of compound content did not differ between diseased infected plants and healthy plants. This causes the strong stimulus as attractant for *D. citri* to come and stay on CVPD symtomatic plants. The intensity of stimulus was increae in line with the increase of disease severity. The number of *D. citri* gravid female was highest (10.90 ± 0.50) on the most severed citrus plants (score 4). It was significantly higher to plants with disease severity score 3 (8.40 ± 0.45), 2 (5.20 ± 0.25), and 1 (2.90 ± 0.23).

D. citri gravid females was more attracted to citrus buds on plants with higher CVPD symptom scores, however the number of eggs laid per bud was significantly higher on healthy plants (Table 2.). Volatile compounds with specific types and intensities were used by *D. citri* as cues to determine the host plant location (Poerwanto *et al*., 2008; Poerwanto, 2013). CVPD decreased the number of eggs laid per bud. The number of eggs laid on healthy and CVPD symptomatic plants was 22.60 ± 0.27 and 9.68 ± 0.47 per bud respectively (Table 2.). CVPD attacks will also cause changes in plant conditions, both in appearance and nutritional content. CVPD attacks cause symptoms of chlorosis, leaves grow stunted, harden and erect and fall off easily (Chung 1987 & Dwiastuti *et al*. 2003). There is also damage to the phloem accompanied by a buildup of starch. Both symptoms and damage increase with the severity of the disease attack (Gonzales 1987).

Table 2. Average number ± SE of *D. citri* (psylids) gravid female stay, and average number of eggs laid per bud on healthy and CVPD symptomatic citrus plants

Plant condition/score of CVPD	Number of psyllids stay/bud	Number of eggs laid/bud
Healthy plant	2.60 ± 0.27 y	22.60 ± 0.27 x
CVPD symptomatic plant	6.85 ± 0.52 x	9.68 ± 0.47 y
P	0.0001	0.0001
1	2.90 ± 0.23 d	5.30 ± 0.21 c
2	5.20 ± 0.25 c	9.90 ± 0.55 b
3	8.40 ± 0.45 b	11.40 ± 0.45 a
4	10.90 ± 0.50 a	12.10 ± 0.38 a
P	0.0001	0.0001

Citrus plants in the budding phase, there was no difference in the appearance of the buds. Buds of healthy plants and of plants with CVPD symptoms showed the same appearance. The typical symptoms of a new CVPD attack appear after the leaves are fully opened. The differences number of eggs laid indicated that the selection of buds by *D. citri* gravid female was more based on age and buds hardness in relation to their ability to lay eggs and feed for the next generation of nymphs.

Insects will perch, feed and lay their eggs on the plants they prefere and provide the necessary nutrients for both themselves and their offspring. The availability of nutrients is strongly influenced by the amount, composition and whether or not it is easily taken up by insects. In plants with CVPD symptoms, the phloem tissue is damaged, resulting in a buildup of nutrients in the leaves. However, the results of research by Wan & Barbosa, 1990) showed that the protein content of tobacco plants did not differ between plants that were attacked by TMV disease and those that were healthy.

The results of the repellent test by using Y-tube olfactometer was shown in Figure 1 up to 3. Significantly higher number of psylids observed in comparison I up to III moved towards the source of host plant volatiles aroma (citrus leave extract) than towards the source of citrus + guava shoot (Figure 1, 2, 3.). This phenomenon showed that the Y-tube olfactometer was an effective apparatus for testing responses of psylid to host plant volatiles aroma. The response of psylids also showed that some psylids did not move to both aroma sources (NM). This response proved that the effect of guava leaf shoots is also able to prevent psylids from choosing citrus leaf extract as a cue for its host plant.

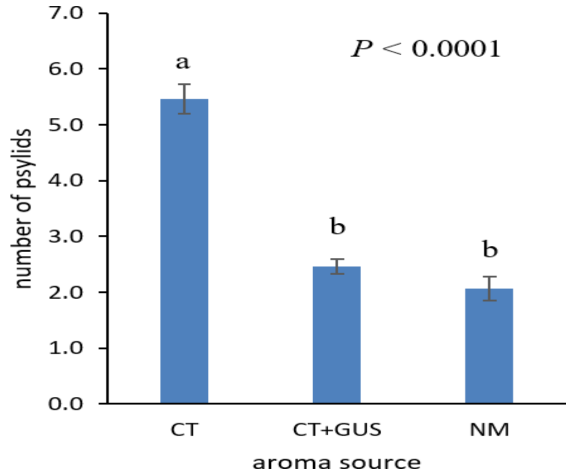

Figure 1. Avarage number ± SE of psylids move to aroma sources of citrus (CT), citrus + guava upper shoot (CT+GUS), and not move (NM) either to CT or CT+GUS

The observations in comparison I showed that the number of psylids moved to the source of the aroma of citrus + guava upper shoot was significantly lower (5.47 ± 0.22) compared to sources without aroma of guava shoots or only aroma of citrus shoots (2.47 ± 0.17) (Figure 1). The same repellency effect also found by Barman and Zeng (2014) and Barman et al, (2016) on guava leaf extract when sprayed to citrus leaves. The existing of the aroma of non host leaves can reduce the population of adult *D. citri* in citrus leaves (Onagbola *et al*, 2011; Poerwanto *et al*, 2008).

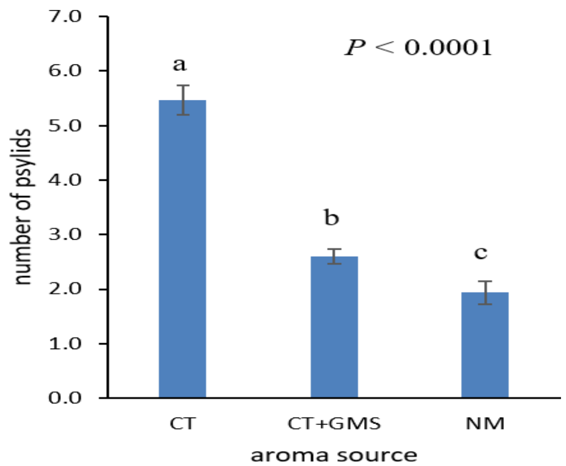

Figure 2. Avarage number ± SE of psylids move to aroma sources of citrus (CT), citrus + guava middle shoot (CT+GMS), and not move (NM) either to CT or CT+GMS

In the comparison II, the same phenomenon also happen on citrus + guava middle shoot. Eventhough more psylids moved towards to the aroma source of citrus shoots without additional guava leaves compared to the aroma source of mixed guava middle shoots and citrus leaves, it was proved that the presence of guava middle shoots would repel psylids (Figure 2.). Some of psylids (1.93 ± 0.28) were also confused to choose the aroma source, and not even moved either to citrus or citrus + guava middle shoot aroma source. The number of psylids moved to the citrus aroma source was 5.48 ± 0.21 significantly different ($P < 0.0001$) compared to the aroma source citrus + guava middle shoot (2.60 ± 0.13).

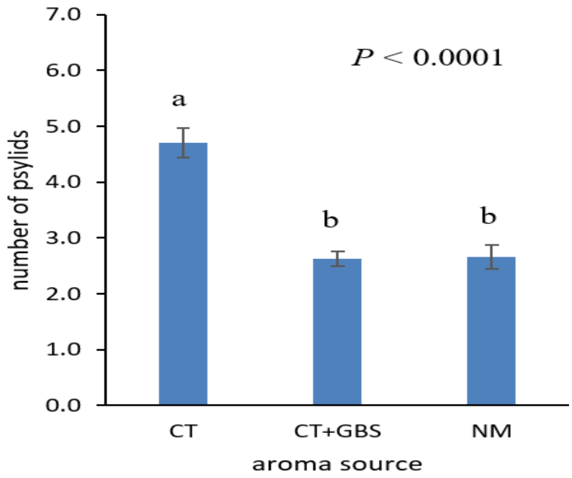

Figure 3. Avarage number ± SE of psylids move to aroma sources of citrus (CT), citrus + guava bottom shoot (CT+GBS), and not move (NM) either to CT or CT+GBS

It seem that the effect of guava bottom shoots in comparison III, tended less than upper shoot and middle shoot (Figure 3.). Less psylids attracted to move to citrus aroma source, eventhough it was significantly higher (4.71 ± 0.23) than citrus + guava bottom shoot (2.63 ± 0.32).

The results showed that guava leaf shoots had repellency effect on psylids. Dose dependent effect was suspected on the repellency activities (Barman and Zeng, 2014). It was suspected that the dose or the volatility of repellent constituen reduced with the increase of the age of leaf shoots (Poerwanto *et al.*, 2020). Psylids uses specific volatile compounds and with a certain intensity as cues to find the location of their host plants, and find parts of plant that are clear from other competitor insects (Zaka *et al*, 2011; Poerwanto, 2013). Specific volatile compounds contained in guava shoots can be used as repellent compounds for *D. citri*. Various of volatile compounds is produced by guava fruit and leaves, such as several sesquiterpenes (Sagrero *et al.*, 1994; Ogunwande *et al.*, 2003), aldehyds and alcohols (Soares *et al.*, 2007). The repellency of these volatiles is highly dependent on their concentration in the air (Zaka *et al*, 2011). Dimethyl disulphide compounds produced when guava leaves are injured also have a repellent effect on *D. citri* (Onagbola et al, 2011).

CONCLUSION AND FURTHER RESEARCH

CVPD symptomatic citrus plants was more attractive to gravid female psylids for laying eggs, however the number of eggs per buds was higher on the healthy citrus plant. The use of guava leaf extract was able to cause a repellent effect on *D. citri* adults. Guava leaf extracs is the prospective alternative mean for controlling CVPD vector and decreasing the disease attack.

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