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DYNAMIC POPULATION OF CITRUS PSYLLID *Diaphorina citri*Kuw. AND THE INCIDENCE OF HUANGLONGBING (HLB) ON INTERCROPPING OF TANGERINE AND GUAVA

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ABSTRACT

The use of botanical substances to encountercitrus psyllid in the framework of integrated HLB control in citrus has been studied. Intercropping tangerine and guava were expected to reduce psyllid visit on tangerine plants and also incidence of HLB. The objective of this research was to evaluate the intercropping of tangerine and guava in relation to the presence of *D. citri*Kuw. and HLB infection. The research was conducted from January 2016 to June 2019 and was designed to facilitate 3 planting types between tangerine and guava, i.e. intercropping of tangerine with guava 'Sukun', intercropping of tangerine with guava 'Jakarta' and monoculture of tangerine. The results showed that the population of psyllid seasonally fluctuated accordingly with the availability of the tangerine juvenile shoots. Along the observation periods; the psyllid population was lower under intercropping tangerine with guava than monoculture tangerine. The visual symptom of HLB was detected after 22 months planting and most of the visually suspected shoots were confirmed by PCR. After 25 months planting, the disease intensity decreased from 18.95 to 4.16% in intercropping tangerine with guava 'Jakarta' and 18.95 to 2.08% in intercropping of tangerine with guava 'Sukun'. In terms of area infection, the suppression of HLB was 65% in intercropping tangerine with guava 'Jakarta' and 77% in intercropping tangerine with guava 'Sukun', when compared to monoculture tangerine. This study demonstrated that HLB intensity and area infection were lower in the intercropping of tangerine and guava than monoculture of tangerine.

Keywords: citrus, *diaphorinacitri*, guava, huanglongbing (HLB), intercropping.

INTRODUCTION

Citrus Vein Phloem Degeneration (CVPD) or Huanglongbing (HLB) is one of the most devastating disease attacking citrus plantation around the world, being present in more than 40 countries (Canales *et al*., 2016), including Indonesia (Nurwahyuni *et al*., 2015). The disease is caused by a non-cultured gram negative α-proteobacteria *Candidatus Liberibacter asiaticus*in Asian countries and related species, *Ca. L. africanus* and *Ca. L. americanus* and *Ca. L. americanus* are the causal agents in Africa and the Americas, respectively(Uechi *et al*., 2019). The pathogens are transmitted by two species of oligophaguscitrus psyllids, *Diaphorinacitri* Kuwayama (Asian citrus psyllid: ACP) and *Triozaerytreae* (del Guercio) (African citrus psyllid) (Lee *et al*., 2015). HLB infected plants exhibit blotchy mottle leaves, stunted growth, yellow shoot, reduced fruit size, corky vein, root decline and ultimately dieback (Pandey & Wang, 2019).

All commercial citrus varieties are susceptible to HLB, though the degree of infection and yield losses were reported to be genotype-dependent (Ramadugu *et al.,* 2016). During 1960's to 1970's, the yield loss of citrus orchards reached 100% in South Africa, China and Thailand and more than 3 million citrus trees in Indonesia became diminishingly unproductive due to the disease infection (Nurhadi, 2015; Rustiani *et al*., 2015). Management in citrus-producing areas without HLB or with low HLB incidence relies on quarantine measures, psyllid control, removal of diseased trees, and replanting with HLB-free trees. HLB inoculums removal is primarily based onvisual observation of disease symptoms and confirmation with quantitative polymerase chain reaction (qPCR). However, the infected plants remain asymptomatic for several months to years, making the symptom-based diagnosis ineffective for inoculum removal (Hu *et al.,* 2018).

The use of synthetic pesticides has been widely implemented to control the transmitter insect, but considered costly, hazardous to environment and not preventing the spread of the disease. The application of botanical control, like trap or repellent plants, is one alternative way out to control the psyllid population and spread of the disease since they were planted and maintained along with citrus plants (Qureshi *et al*., 2014). Guava (*Psidium guajava*) has been studied for their potential in reducing the existence of citrus psylla, *D.citri*Kuw. (Barman *et al*., 2016; Zaka *et al*., 2010). The effect of repellent was relied on two putative mechanisms. First, the volatile compounds released by the guava leaves and apical that could be directly repellent to psyllids, and thereby limit the pathogen spread (Barman & Zeng, 2014). Guava leaf volatiles were reported to inhibit attraction of psyllids to normally attractive host citrus volatiles (Qureshi *et al*., 2014) and moreover, the psyllid settlement on citrus was significantly reduced in laboratory cage trials (Zaka *et al*., 2010). Mechanically wounded guava was also found to produce toxic and repellentdimethyl disulphides, while citrus does not, suggesting these, or other guava compounds, may be responsible for the observed deterrence (Onagbola *et al*., 2011; Fancelli *et al.*, 2018).

The other mechanism was that the guava volatiles could be detected by citrus plants, leadingto changes in the characteristics of citrus that mediate host selection. This indirect, or plant-plant communication was hypothesized as aerial cues released by mechanical damage to the plant leaves could cause biochemical changes in conspecific undamagedneighbors, and which reduced herbivore performance, including insect pest (Bouwmeester *et al.*, 2019). These results have since been supported in other systems by several studies demonstrating that volatile-based, plant-plant communication can cause associational resistance (Coppola *et al.,* 2017; War *et al.*, 2011; Thakur & Sohal, 2013). Based on the said details, the intercropping of citrus and guava might interfere *D. citri*Kuw. behavior to citrus plant. These insect pests were expected to be repelled due to the volatiles released from the guava. These conditions would not only reduce the plant damages caused by the insect attacks, yet diminish the HLB incidence due to less vector activity as well. The research was intended to evaluate the intercropping of tangerine and guava in relation to the presence of *D. citri*Kuw. and HLB infection.

MATERIAL AND METHODS

The research was conducted from January 2016 to June 2019 at Pekutan, Bayan, Purworejo, Central Java, Indonesia at the altitude of 50 masl. The area is well known as one of the main citrus production centers in the province. The tangerine accessions used in the study was 'Banjar' while the intercropped guava plants were 'Jakarta' and 'Sukun'. The research was arranged in a non-factorial experiment with 6 replicates. The designed treatments were (1) intercropping of tangerine 'Banjar' with guava 'Sukun', (2) intercropping of tangerine 'Banjar' with guava 'Jakarta' and (3) monoculture of tangerine 'Banjar'. The planting layout of each treatment was presented in Figure-1.

▲	▲	▲	▲	▲
O	O	O	O	O
▲	▲	▲	▲	▲
O	O	O	O	O
▲	▲	▲	▲	▲

(a)

x	x	x	x	x
O	O	O	O	O
x	x	x	x	x
O	O	O	O	O
x	x	x	x	x

(b)

O	O	O	O	O
O	O	O	O	O
O	O	O	O	O
O	O	O	O	O
O	O	O	O	O

(c)

O = Tangerine 'Banjar' ▲= Guava 'Jakarta' x = Guava 'Sukun'

Figure-1. Planting layout of intercropping between (a) tangerine 'Banjar' and guava 'Jakarta', (b) tangerine 'Banjar' and guava 'Sukun', and (c) monoculture of tangerine 'Banjar'.

The 8 months guava seedlings of both varieties and tangerine were planted at the same time. Prior to planting, the planting hole was made with the size approximately of 60 x 60 x 60 cm. Each planting point was fertilized by a mixture of 5 kg manure, 200 g NPK (16: 16: 16) and 5 kg of top soil. The planting holes were arranged 1.5 m among others and the number of planting holes/number of planted plants in each treatment was 25 plants (Figure-1). The distance between treatments was 2.5 and between replications was 2.5 m. After the tangerine and guava seedlings were planted, for about 5 l of water was given to each planted seedling to facilitate humidity. The plants were then maintained by watering twice a week or when necessary. The pest and disease control were implemented by pesticide sprays once a week until harvesting period. Additional fertilizers using 300 g (16:16:16) NPK per plants were applied every 4 months and 5 kg manure per plant in every 6 months. The height of guava plants was maintained shorter than tangerine plants to reduce space and light competitions.

Variables observed were citrus psyllid population, HLB incidence, and HLB development after 24 months planting. Aside from the visual symptom, the detection of HLB infection was performed using PCR using primer16S rDNA, forward primer OI1 and reverse primer O12c(Taufik *et al.*, 2010). The disease intensity was calculated based on Meitayani *et al.* (2014).

$$I = \frac{\sum (n\ x\ v)}{Z\ x\ N}\ x\ 100\%$$

Where:

- I = Intensity of HLB infection (%)
- v = Scale of the observed damage
- n = Number of infected plants that categorized in the respected damage scale
- Z = Highest scale of the observed damage
- N = Total number of observed plant samples

Table-1. Scale and damage criteria of HLB infection on citrus (Meitayani *et al*., 2014).

Scale	Damage Criteria
1	Not infected (symptomless).
2	Low, the intensity ranges 1 - 25% of the observed apical flushes.
3	Medium damage, the intensity ranges 25 - 50% of the observed apical flushes.
4	Heavy damage, the intensity ranges 50-75% of the observed apical flushes.
5	Very heavy damage, the intensity ranges 75 - 100 % of the observed apical flushes.

The category of HLB intensity was determined based on Ahmad *et al*., (2011) and presented in Table-2.

Table-2. Level of HLB intensity on citrus.

Damage scale	Disease intensity
0%	Not infected (symptomless)
>1% - 25%	Low
>25% - 50%	Medium damage
>50% - 75%	Heavy damage
>75% - 100%	Very heavy damage

The disease incidence was calculated using the following formula.

$$L = \frac{n}{N} \times 100\%$$

Where,
L = Disease incidence/area infection
n = Number of infected plants
N = Number of observed plants

RESULTS AND DISCUSSIONS

The tangerine and guava plants were planted in the end of October 2016 after land preparation. These schemes were arranged since the period was already in the early rainy season. In rainy season, the soil and air relative humidity were high, and such condition was expected to reduce environmental stresses to the newly planted young plants. Water availability in the soil was also considered abundant, thus the supply of water in root zones was also sufficiently fulfilled (Ashari *et al*., 2014).

***D. Citri*kuw. Population**

The existence of citrus psyllid (D. citriKuw.) was initially observed after 6 months planting, though the detected insects were still rare. The appearance of the D. citriKuw. became more obvious, yet still considered low until 12 months after planting (Figure-2). The rare appearances of citrus psyllid during the first 12 months after planting were predicted to have relation with the growth stage of tangerine plants. The visiting insect pest into host plants and insect migration was determined by several factors like host preferences, thermal requirements, and responses to visual and chemical volatile stimuli. However, it is very likely that shoot characteristics and nutritional quality may dictate the reproductive potential of D. citri including mating, egg maturation and successful immature development (Cifuentes-Arenas *et al.*, 2018). *Diaphorinacitri* often exhibit distinct morphological changes during growth and development. Such ontogenetic changes are typically associated with variations in physicochemical properties such as texture, chemical composition and volatiles released from leaf tissues. Adult D. citri preferentially selected young shoots for feeding, since young flush shoots had higher concentrations of macro and micro nutrients relative tomature ones (Sétamou *et al*., 2016). Thus, the low visiting psyllid during the first 12 months in all evaluated tangerine plants were related with the absence of nor low young leaf flushes on the plant. On the following rainy season (November 2017), the plants started the newly juvenile stage and formed flush leaves. Volatiles released by the flushes attracted more adult psyllids (since only adult stage that can migrate/move) on the tangerine plants and the number of observable insects became obvious in the following months, especially on monoculture tangerine.

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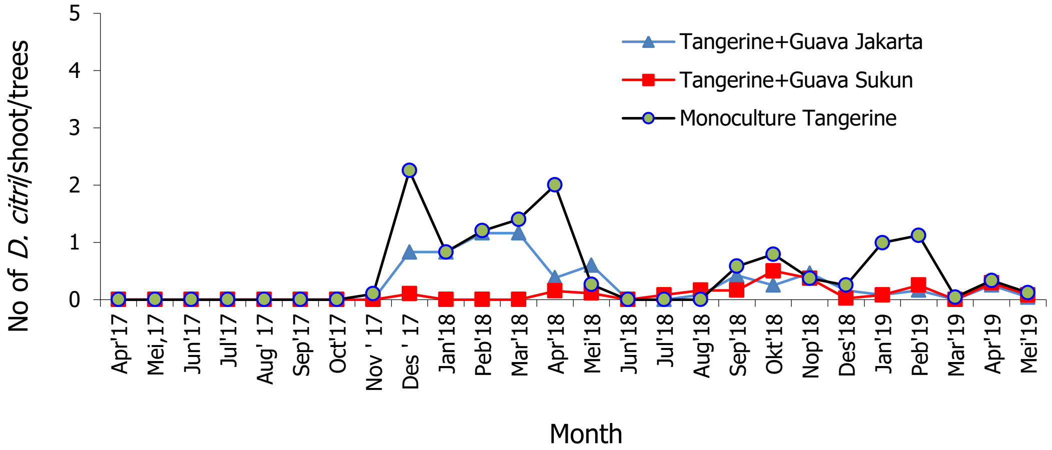
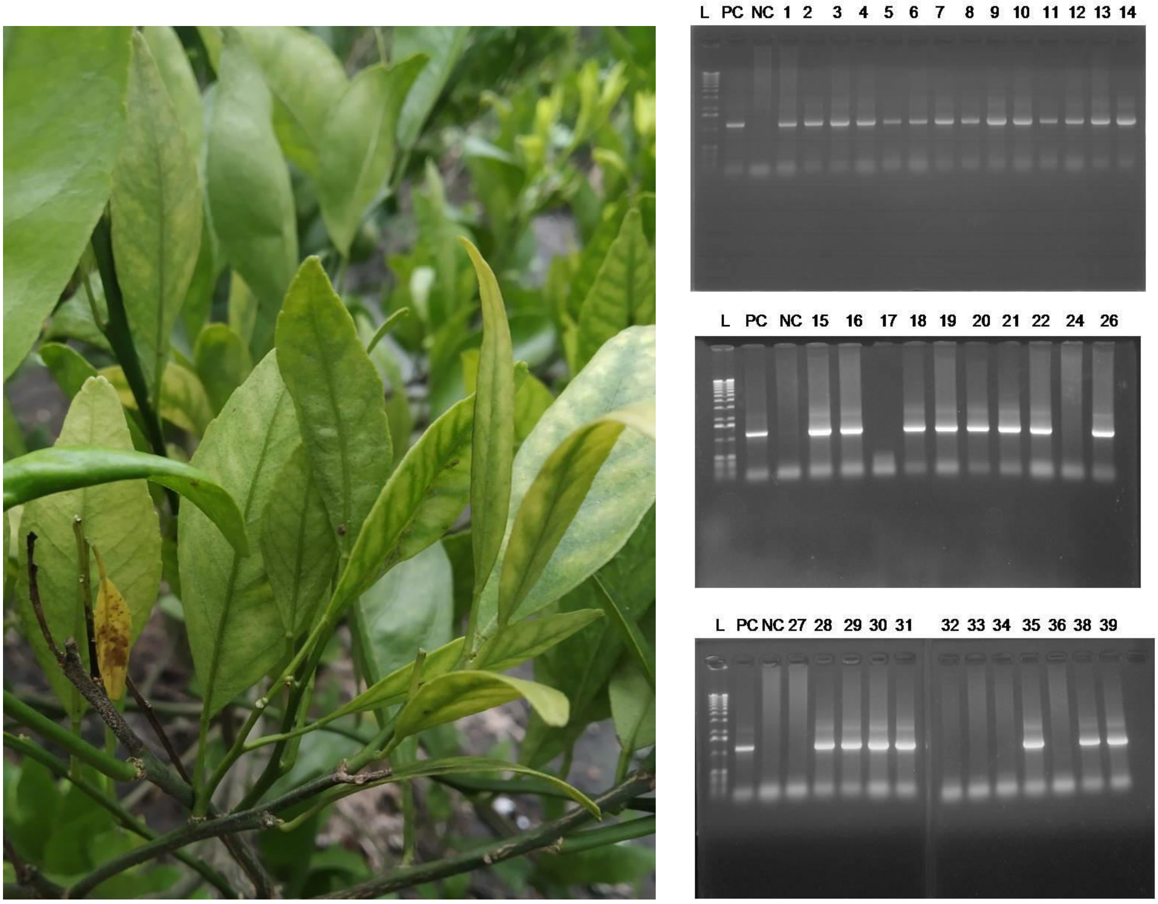


Figure-2. Population dynamic of D. citrionmonoculture tangerine and tangerine intercropped with guava.

After 12 months planting, the population of adult psyllid fluctuated up to 31 months after planting. On rainy seasons (November to April), the population of adults were detected high and diminished during dry season (May to October) (Figure-2). Thissituation was related with the availability of leaf flushes as the source of food. During rainy seasons, tangerine plants exhibited juvenile stage and produced new young leaves. These abundant source of food induced the insect fecundity to produce more offspring and attract more adults from other plants (Johnston *et al*., 2019)surrounding the planted tangerine. While during dry season or when the flushes had turned into more mature stages, the developmental stage of insect was no longer in the nymphal stage and the mature insects migrated to find another source of food (Teck *et al*., 2011). Figure-2 also showed that the population of citrus psyllid in the intercropping tangerine with guava 'Sukun' was the lowest, followed by the intercropping tangerine with guava 'Jakarta', and the monoculture tangerine was the highest during the observation periods. The lower citrus psyllid in the intercropping tangerine and guava 'Sukun' were assumed due to the faster growth rate of guava 'Sukun' than guava 'Jakarta'. The larger leaf area surface of guava 'Sukun', then might produce more repellent volatiles. The lower psyllid in tangerine intercropped with guava than monoculture tangerine also indicated that the existence of guava plants enabled to interfere psyllid normal attraction to host/tangerine plants (Poerwanto & Solicah, 2019; Zaka *et al*., 2015).Growing guava (*Psidium guajava*) as an intercrop appeared to be a potential means of reducing number of psyllids within citrus orchards.

Detection of HLB Incidence

The HLB incidence on tangerine plants was initially detected on July 2018 (22 months after planting). The incidences were detected in all cropping types treatments, yet in different intensities. The HLB infection was characterized by yellowing of an individual leaf or in one cluster shoot (Figure-3a). The leaves that turned into yellow due to HLB infection then showed a symmetrical blotchy yellowing or mottling between the leaf veins, with patches of green on one side of the leaf and yellow on the other side. Shen *et al*., (2013) and Whitaker *et al*., (2014) furtherly stated that in severe attacks, the fruits size becomes smaller, and the juice turns bitter. The fruit may remain partially green and becomes lopsided, has dark aborted seeds, and tends to drop prematurely. Chronically infected trees are sparsely foliated with small leaves that point upward, and the trees have extensive twig and limb dieback. Eventually, the tree stops bearing fruit and dies.



(a) (b)

Figure-3. (a)HLB symptoms detected on leaves of tangerine 'Banjar' after 22 months planting and, (b) the PCR analysis of tangerine DNA to confirmthe HLB infection.

For about 39 suspected HLB symptomized shoots from different tangerine plants were collected and assessed for further confirmation of HLB infection using PCR. The bands of HLB infected samples were observed on DNA size of 1160 bps. From 39 samples, 32 samples were classified as positively infected and the other 7 were negative (Figure-3b). The successful detection of PCR for confirmation of HLB infection in tangerine have also been reported by Nurwahyuni *et al.,* (2015), Taufik *et al*., (2010), and Meitayani *et al.*, (2014).

HLB Intensity and the Area Infection

The observation of HLB incidence on 22 months after planting revealed that the disease intensities were varied among the cropping types. The highest disease intensity was detected on tangerine monoculture (13.33%), followed by intercropping tangerine with guava 'Jakarta' (3.3%) and tangerine with guava 'Sukun' (0.63%). Similar trends were also observed in area infection, in that tangerine monoculture was still to be the highest, followed by intercropping of tangerine and guava 'Jakarta' and tangerine and guava 'Sukun'. The disease intensity and area infection of HLB on the respected tangerine planting types after 22 months planting were presented in Figure-4.

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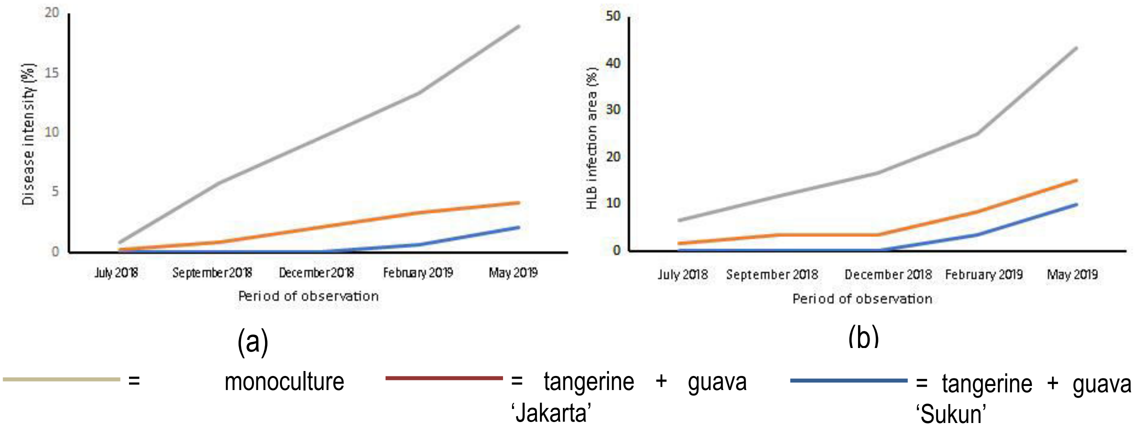

Figure-4. (a) HLB disease intensity and (b) area infection on tangerine planted in different planting types from 22 to 32 months after planting.

Figure-4 showed that intercropping tangerine with guava lowered HLB intensity and area infection after 22 months planting. The reduction of disease intensity was 75% and 95% from intercropping tangerine with guava 'Jakarta' and tangerine with guava 'Sukun', respectively compared to monoculture tangerine. In terms of area infection, the suppression of HLB was recorded 67% and 87% from intercropping tangerine with guava 'Jakarta' and tangerine with guava 'Sukun', respectively compared to monoculture tangerine. The suppression of HLB spread was more obvious after 25 months planting. The reduction of disease intensity was 78% and 89% from intercropping tangerine with guava 'Jakarta' and tangerine with guava 'Sukun', respectively compared to monoculture tangerine. In terms of area infection, the suppression of HLB was recorded 65% in intercropping tangerine with guava 'Jakarta' and 77%% in intercropping tangerine with guava 'Sukun', when compared to monoculture tangerine.

The lower HLB disease intensity and area infection in intercropping tangerine and guava 'Jakarta' and tangerine with guava 'Sukun' than monoculture tangerine indicated that the suppression of HLB spread were connected with the lower population of citrus psyllid in the respected sites. This indirect relationship inferred that the spread of HLB could be partly inhibited through the control of psyllid. The existence of guava plants among the tangerine enabled reduced the visiting psyllid, thus lowering the disease intensity and area infection. The results suggested that intercropping citrus and guava may become a part of integrated and comprehensive programs and activities of the control of disease vectors, aside from planting the trusted and healthy seedlings and the control of HLB sources (Abdullah *et al.*, 2009).

CONCLUSIONS

The visiting citrus psyllid (*D. citri*Kuw.) was initially observed after 6 months and became obvious after 12 months. In all planting types, the population then seasonally fluctuated in line with the availability of juvenile shoots. The existence of guava plants among tangerine plants reduced the psyllid visit in tangerine. Visual symptom of HLB was detected after 22 months planting and 82.1% of the visually symptomized shoots were positively infected as confirmed by PCR. HLB intensity and area infection were also lower in the intercropping of tangerine and guava. After 25 months planting, the reduction of disease intensity was 78% and 89% from intercropping tangerine with guava 'Jakarta' and tangerine with guava 'Sukun', respectively. While in terms of area infection, the suppression of HLB was recorded 65% in intercropping of tangerine and guava 'Jakarta' and 77% in intercropping of tangerine and guava 'Sukun', compared to monoculture tangerine.

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