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MANAGEMENT OF CVPD BY CONTROLLING *Diaphorina citri* FOR DEVELOPING AGRO-TOURISM ON CITRUS

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ABSTRACT

Indonesia is the world citrus producer with more than two hundreds varieties of citrus. The varieties are locally specific which are potential for agro-tourism. Most of citrus orchards have been destroyed by Citrus vein phloem degeneration (CVPD). CVPD is the most impediment disease in citrus production in the world. It mainly vectored by Diaphorina citri Kuwayama. The disease also caused some of Indonesian indigenous citrus varieties endangered. Intensive application of insecticides was ineffective and also costly. Comprehensive strategy for managing CVPD is being conducted and evaluated by the integration of planting disease free seedling, and controlling disease vector (D. citri). The controlling strategy consists of guava intercropping, alternative host sanitation, planting area isolation, and mineral oil application. The strategy would decrease the infection of CVPD by reducing the inoculums titer, eliminating contacts between disease inoculums with the vector, and decreasing infective vector population. Conservation of Indonesian locally specific citrus varieties will develop agro-tourism on citrus

KEYWORDS: Citrus, CVPD, D. citri, guava intercropping, mineral oils

INTRODUCTION

Indonesia is currently the second world citrus producer after China with more than 210 varieties of citrus. Most of them are unique and locally specific. They have been supporting tourism and potentially for developing agro-tourism on citrus. The citrus production in 1995 was 143,059 ton, and it decreased to 91,469 ton in 1996. The production increased into 696,422 ton in 1997 and decreased sharply into 449, 552 ton in 1999. Recently, citrus production in Indonesia has developed rapidly. In 2007, the production increased to 2,625,884 ton. In 2008, the production decreased again into 2,467,632 ton and in 2010 were 2,032,665 ton. It was due to the orchard extension (BPS, 2011). Fluctuation on citrus production was mainly caused by Citrus vein phloem degeneration (CVPD). The disease caused 50 million citrus trees lost both in South Asia and South East Asia CVPD was first reported in Indonesia in 1964 (Semangun, 1991). In Indonesia the most susceptible cultivated form of citrus to CVPD is *C. reticulata* Blanco (Rutales: Rutaceae). Severe epidemics of the disease occurred in the 1960s, especially in Java and Sumatera where at least 3 million trees were destroyed. It was informed that the disease has destroyed 9 million out of 42.8 million total citrus-trees in Indonesia in 1990. The economic loss was about 35 billion rupiah per year (Nurhadi, 1991). Many highly productive plantations where yields were 20 ton per ha decreased sharply into 8.6-15 ton per ha per annum (Irawan *et al.,* 2003). A citrus rehabilitation program based on the eradication of infected trees and the production and use of

disease-free bud wood by shoot-tip grafting was initiated in the mid 1980s. However, the citrus trees in Indonesia have not been free of CVPD (Bove *et al.,* 2006). CVPD continues to be the major impediment to citriculture in Indonesia.

CITRUS

Citrus is considered originated from Southeast Asia between India and China and southwards through Malesia (Dugo & Di Giacomo, 2002). Citrus was spread from the Far East to Middle East, Mediterranean, and eventually America. The genus Citrus belong to the Plantae Kingdom, Spermatophyta Division, Angiospermae Sub division, Dicotyledoneae Class, Rutales Order, the Rutaceae family. There were six genus belong to the Rutaceae family: 1) *Citrus*; 2) *Microcitrus*; 3) *Fortunella*; 4) *Poncirus*; 5) *Cymenia*, dan 6) *Eremocitrus* (Martasari & Hardiyanto, 2003). Recent work suggests that the genus *Citrus* comprises about 25 species (Mabberley, 2004). They are: *C. medica*, *C. australis*, *C. australasica*, *C. garrawayi*, *C. glauca*, *C. gracilis*, *C. inodora*, *C. neocaledonica*, *C. oxanthera*, *C.* sp. (*Oxanthera fragrans*), *C. undulata*, *C. polyandra* (syn. *Clymenia*), *C. warburgiana, C. wintersii* (syn. *Microcitrus papuana*), *C. cavaleriei* (*C. ichangensis*), *C. japonica* – kumquats (*Fortunella*), *C. reticulata*, *C. trifoliata* (syn. *Poncirus*), *C. maxima* – pomelo, *C. amblycarpa* (a possible hybrid), *C. halimii*, *C. hystrix* – kaffir lime (*C. macroptera*), *C. warburgiana, C. swinglei*, *C. latipes*.

Some citrus in Indonesia which are developed, such as *C. reticulata*: SoE mandarin from NTT; Batu 55 mandarin from East Java; *C. sinensis*: Madu siem, Maga siem and Beras Sitepu from Medan; Pontianak siem from West Kalimantan; Pacitan sweet orange from East Java; Waturejo sweet orange from Central Java; *C. maxima*: Nambangan Pummelo, Sri Nyonya Magetan from East Java; *C. aurantifolia* and *C. hystrix* : "Nipis"; and *C. medica*: "Lemon" (Agisimanto & Sutarto, 2007).

CVPD

CVPD, also recognised as Huanglongbing or greening disease, was firstly found and produced a serious problem in the Chaozhou/Shantou region of Guangdong in the mid 1930s (Lin, 1956). The disease epidemic was found in some Asian countries (Tirtawidjaja *et al.,* 1965; Garnier & Bové, 2000; Weinert *et al.,* 2004), and in the Arabian Peninsula (Bové & Garnier, 1984). It was also found in Africa, Mauritius and Réunion (Garnier *et al.,* 1996), United States of America (Florida and Louisiana), Cuba (Martínez *et al.,* 2008), and Brazil (Teixeira *et al.,* 2005). The disease caused 50 million citrus trees lost both in South Asia and South East Asia (Gonzales, 1987).

At the initial stage trees develop mottle leaves in one or few branches similar to that caused by the lack of zinc (Dwiastuti *et al.,* 2003). Leaves become smaller and bunchy. In most cases, the disease appeared localized on the tree. In severe cases, all cultivars of *C. reticulata* suffered from dieback of branches, mottling or yellowing of leaves were pronounced and extensive: the leaves become reduced in size, grow in an upright position and become vein corking. Trees will suffer from premature yellowing of fruit, undersized fruits that fall before maturity, browning of the stem and dieback exhibited by the branches. The fruits become bitter and sour and the sugar concentration decreases. In subtropical climates, CVPD induces heavy leaf shedding at the beginning of the winter season (Gonzales, 1987). CVPD agent is a fastidious or unculturable Gram-negative bacterium with the proposed name '*Liberibacter asiaticus*' and '*Liberibacter africanus*' for Asian and African types, respectively (Nakashima *et al.,* 1998). The hosts of CVPD agent are not only citrus, but also *Catharanthus roseus*

(periwinkle), *Cuscuta campestris* and *Murraya exotica* also. The latent period in citrus plants before symptoms is expressed ranges from four months to one year or more. Once the citrus plant has been infected, it will die in two to four years (Su & Huang, 1990). There were 27 species and hybrid of Rutacea genus which could become the host of CVPD i.e. *Atalantia*, *Citrus* (including *Fortunella* and *Poncirus*), *Murraya* (*M. paniculata*) in Citreae, *Bergera* (*B. koenigii* or *Murraya koenigii*), and *Clausena* in Clausenaeae. The most devastated commercial citrus is *C. medica*, followed by *C. reticulata* (Yang *et al.*, 2006).

CVPD is not transmitted by seeds, but is naturally transmitted through vegetative propagation and by insect vector (Subandiyah *et al.*, 2000). '*Candidatus* L. africanus' is transmitted by insect vector of *Tryoza erytreae* while '*Candidatus* L. asiaticus' and '*Candidatus* L. americanus' are transmitted by *D. citri* (Bove, 2006). The pathogens have been detected in haemolymph and salivary glands of *T. erytreae* and *D. citri* (Xu *et al.*, 1988).

VECTOR OF CVPD (*Diaphorina citri*)

D. citri Kuwayama (Homoptera: Psyllidae) in Asia was spread in the Arabian Peninsula (Saudi Arabia and Yemen), and from Afghanistan through the Indian Subcontinent, Southeast Asia and East Asia (the Ryukyu Archipelago, Okinawa and Kyushu in Japan, in China, particularly Taiwan and the coastal provinces of Guangxi, Guangdong, Fujian and Zhejiang), the Philippines and through the Indonesian archipelago to north eastern Papua New Guinea (Bellis *et al.*, 2005). In America it was distributed in Hawaii, Guam, Alabama, California, Florida, Georgia, Louisiana, Mississippi, South Carolina, Texas, Commonwealth of Puerto Rico, Bahamas, Cayman Islands, Cuba, Jamaica, Dominican Republic, Guadeloupe, Venezuela, Brazil, Paraguay, Uruguay and Argentina; the Mascarenes (Mauritius and Réunion) (Halbert & Manjunath, 2004).

Female psyllids lay eggs only in the growing tips on the new shoots of the host plant of young host plants, preferring flush growth < 6 mm in length. Numbers of eggs laid on a flush decline rapidly as the length of flush increases, and when individual leaves attain lengths > 10 mm, and flushes 50 mm (Leong, 2006). The population density of eggs and nymphs is entirely dependent on the availability and abundance of new plant shoots (Tsai *et al.*, 2002). Most eggs are laid within 14 days of new growth commencing (Lin *et al.*, 1973). They suck phloem sap from juicy leaves or petioles, and excrete white pellets or threads. It takes 15 days to complete the five nymphal instar stages and adults can live for up 6 to 9 months (Xie *et al.*, 1989). Flight is limited to 0.5-2 km when searching for a host; dispersal over 90 to 470 km is, or may be, possible in strong winds, such as those associated with cyclones (Halbert *et al.*, 2008).

Only adult psyllids and the 4-5th instar nymphs are able to acquire the pathogen (Capoor *et al.*, 1974; Xu *et al.*, 1988). However, Hung *et al.* (2004), using PCR, detected the pathogen in second to fifth instar nymphs and adult *D. citri*. After feeding on a diseased plant for 30 minutes or longer, the pathogens remain latent inside the vector from between 3-20 days (Chavan, 2004). After acquisition from an infected plant, the pathogen multiplied first in the midgut epithelial cells of *D. citri*, and then penetrated through the basal lamina and entered into the haemocoel, where it multiplied again, before invading haemocytes, nerves tissue, fat body, trachea, muscle and eventually the salivary gland (Jiang, 2005). Once the psyllid vector acquires the pathogens, it can transmit them throughout its life span. However, it can not transfer the virulence to its progeny via eggs (Xu *et al.*, 1991; Hung *et al.*, 2004). The disease may shorten the age

of female, decrease fecundity, survivorship of second instars nymph, and the intrinsic rate of increase; and increase the ratio of male/female (Poerwanto, 2000; Jiang, 2005).

CONTROL STRATEGY OF *D. citri*

Integrated strategy for controlling CVPD and conserving endangered citrus varieties can be developed by mineral oil application, guava intercropping, alternative host sanitation, and planting area isolation. It also contributed in reducing highly poisoned pesticides contamination in ecosystem, especially in the area of citrus orchards.

Oils application

HMOs and AMOs (Horticultural and Agricultural Mineral Oils) are highly refined mineral oils derived from crude petroleum oils. They are paraffinic (≥ 60% of carbon atoms occur in chains). HMOs are called narrow-range petroleum spray oils and AMOs are called broad-range petroleum spray oils. Common median *nCy* values of HMOs are *nC*21 and *nC*23. For AMOs they are *nC*23, *nC*24 and *nC*25 (Agnello, 2002; Beattie and Hardy, 2005; Beattie, 2009). Those mineral oils are less phytotoxic to the plant and do not induce any carcinogenic effect to human (Beattie, 2010; personal communication)

Application of oils for citrus pests is ranged from 0.4% to 0.5% sprays at 5-14 day intervals within flush cycles (beginning when the first buds in each cycle begin to open) to control a range of pests and diseases, particularly *D. citri* (Poerwanto *et al.*, 2008, 2010) and citrus leafminer, simultaneously. Application of mineral oils to citrus mandarin leaves reduced 56.7% - 61.3% proportions of psyllids attracted to host plant volatiles (Poerwanto *et al.*, 2008). *D. citri* gravid female rejected to lay their eggs on plant treated with mineral oils (Rae *et al.*, 1997). Responses of adult *D. citri* to mineral oil deposits are olfactory and related to detection by antennal receptors of oil volatiles and/or plant volatiles. This outcome indicates that application of the oils to the mandarin leaves may have: (a) suppressed release of attractant host plant volatiles; (b) masked attractant host plant volatiles; (c) led to the release of repellent volatiles from leaves; and/or (d) to adults being repelled by oil volatiles (Poerwanto *et al.*, 2008).

Guava interplanting

Based on observations by Vietnamese ACIAR researcher team, it is suggested that citrus groves interplanted with guava trees citrus was free from *D. citri* invasion and low incidence of HLB-infected trees (Beattie *et al.*, 2006). Certain volatile compounds in guava leaf could be developed as repellents for the management of citrus psylla. Guava fruits and leaves produce a wide range of volatile compounds, such as sesquiterpenes (Sagrero-Nieves *et al.*, 1994; Ogunwande *et al.*, 2003), aldehydes and alcohols (Idstein & Schreier, 1985; Begum *et al.*, 2002, 2004; Soares *et al.*, 2007). Some of these aldehydes and alcohols are the so called 'green leaf volatiles' that have been shown to have repellent effects on insects (Jang & Light, 1991).

The present study revealed that the repellent action of guava against citrus psylla is dose-dependent, with very low doses having little effect on citrus psylla. This result indicates that to control citrus HLB by interplanting guava trees in citrus groves, sufficient numbers of guava trees are needed to keep the dosage of volatile compounds emitted from guava at an effective level in the entire grove (Zaka, *et al.* 2010). In China, observations revealed that even in the presence of guava trees scattered inside or around the groves, citrus psylla populations are high. This could be due to the fact that the guava trees present did not release enough active volatile compounds (Beattie *et al.*,

2006). In Indonesia, ACIAR researcher team interplanted guava among the young citrus plants. The ratio of guava: citrus population is 1:8 up to the next 6 months, there was no *D. citri* and CVPD symptom found in the orchard, whilst CVPD symptoms (was confirmed with PCR) and 0.4 imago of *D. citri* with 0.3 nymph colony and 0.1 egg colony per plant were found in the orchard 100-1000 meters apart away planted of 50-200 citrus trees of 3-6 years old (Pustika *et al.* 2008). In Vietnam, it was suggested that guava trees are interplanted prior to citrus at a ratio of one guava tree to one citrus tree (Beattie *et al.*, 2006).

Alternative host sanitation

D. citri colonies are also found on ornamental plants, such as orange jasmine (*Murraya paniculate* and *Murraya exotica*), and *Bergera koenegii* all year round (Tsai *et al.*, 2002). Four of 16 species of weeds in citrus orchards are able to be the alternative host. There were *Alternathera philoeroides* of grass group, *Amaranthus spinosus, Ludwigia perrenis*, and *Boerhavia erecta* of broadleaf weeds (Hardiastuti & Poerwanto, 2011).

D, citri was able to survive a maximum of eight days with the mean longevity of 5.91 ± 0.251 days on the broadleaf weed *B. erecta*, but was not able to complete one life cycle. It was significantly different to other weeds, *A. spinosus*, *A. philoeroides*, and *L. perrenis* which were survived for 3.57, 0.20, and 0.20 days respectively. The ability to survive in non host plants indicated the existence of nutrients content in the weed that resembles its host plant nutrient content or the existence of certain substances that could stimulate *D. citri* to stay for feeding (Hardiastuti & Poerwanto, 2011). The substances could be a various compounds of alcohol and aldehyde from the leaves of which were specific and volatile used by insect to find its host plant (Visser, 1986). However, the type and number of nutrient content was not as complete as in the host plant for surviving and completing its life cycle. Similar results were also obtained by Sudiono and Purnomo (2008) in Gemini virus isect vectors (*Bemecia tabaci*), and Hardiastono (2001) on Peanut Stripe Virus (PStV). *B. tabaci* were able to live on broadleaf weed *Ageratum conyzoides*. The weeds also serve as a source of inoculum since they were able to be infected by Gemini virus. While *Amaranthus spinosus, Bidens pilosa, Crotalaria incana, Glycine max* and *Physalis angulata* could be as alternative hosts and source of inoculums for PStV infection.

The implications of those survivorships of *D. citri* in ornamental plants and some weed species is the presence of alternative host for *D. citri* when citrus plant is not available as a food source, either because there are no plants or when plants are treated with pesticides. The existence of alternative host will cause the population of *D. citri* is available throughout the season and serve as initial population for the next generation population. The role of *D. citri* as a propagative vector of CVPD pathogen will also make the disease inoculums always available throughout the season in field (Hardiastuti and Poerwanto, 2011). This will further complicate the control measures against CVPD on citrus plant, since the disease transmission is highly dependent on the availability of disease inoculums and insect vector population in the field (Chen, 1998).

Planting area isolation

It had been reported that plant odours (volatile) detection and processing plays an important role in host plant selection by herbivorous insects (Moran and Brown, 1973; Todd *et al.*, 1990; Appleton *et al.*, 2004; Bichao *et al.*, 2005). The volatile information was received by insect's central nervous system at a fine-scale spatio-temporal

resolution (Bruce *et al.*, 2005). Odours vary substantially, depending on species, cultivar, growing conditions, age and plant parts (Takabayashi *et al.*, 1994). Establishing new citrus orchard in the isolated area will delay the *D. citri* infestation and CVPD infection. It takes longer time for citrus plant odours to be detected by *D. citri* and attract its colony to infest.

Initial colony of *D. citri* was found 20 months after planting at new citrus orchard in an isolated area, far from the established citrus orchard and was surrounded by paddy's field. Five months more was needed for the colony of *D. citri* to colonized 100% citrus plants in one block. The colonization of *D.citri* in other blocks started from one plant spread to other plants at the same block before migrating to other nearby blocks (Poerwanto, 2010). The spread was very slow because the flight ability of psyllid was very low. Aubert (1990) and Tsai *et al.* (2002) reported that wind was needed by psyllid in spreading in long distance (0.5 – 4 Km).

CONCLUSIONS

Management of CVPD disease should be comprehensively conducted to conserve local citrus varieties for developing agro-tourism on citrus. It should be started by seeking new area for establishing citrus orchard. The area should be far enough from citrus orchard and free from alternative hosts of CVPD and its vector. Second step is interplanting guava among citrus plants followed by using mineral oils when the vector colony was found. Last but not least is keeping the orchard always clean of weeds which could be used as alternative host by vector.

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