Population dynamics of *Diaphorina citri* with the implementation of integrated management of healthy orange gardens (PTKJS) and CVPD detection with PCR engineering

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**Abstract.** This study was to determine the population dynamics of *Diaphorina citri* with the Application of Integrated Management of Healthy Citrus Gardens (PTKJS) and detection of CVPD by PCR technique. This research was conducted in a village in Tebing Batu village, Sebawi District, Sambas Regency from February to October 2018. The research used observation / monitoring techniques for insect vectors, the abundance and diversity of insects and disease symptoms using the PCR technique. The results showed that the application of monitoring techniques which was continued by PTKJS, there was an increase in crop yields and an increase in the diversity of the highest insect species trapped after the implementation of PTKJS was dominated by the Hymenoptera Order with 17 families out of a total of 7515 trapped insects. The application of monitoring techniques has resulted in an average emphasis of *Diaphorina citri* from 16.33 to 1.67. The PCR method is a very sensitive method and can be used to detect pathogenic bacteria that cause CVPD based on testing of 27 samples repeated 3 times showing > 90% positive indicating the presence of *L. asiaticum* bacterial infection that causes CVPD.

1. **Introduction**

The continuity of orange production in West Kalimantan in general and Sambas Regency in particular is faced with the problem of CVPD which is spread by the insect *Diaphorina citri*. The potential losses caused by this CVPD attack are enormous. In 2007, the decline in productivity due to CVPD infection and the death of sick plants in Sambas Regency has increased. This is indicated by a decrease in production of 20,000 tonnes of citrus fruit or at least equivalent to Rp. 60 billion rupiah each year. And if the productivity of citrus plants infected with CVPD rises to the level of 30-40%, then the losses borne by citrus farmers in Sambas Regency could reach Rp. 120 billion per year, and will continue to increase as the disease develops [3].

On the other hand, the total population engaged in the citrus agribusiness sector in Sambas Regency reaches 23,826 people or about 20% of the population of Sambas Regency. This means that around 10,000 people will lose their livelihoods in the citrus agribusiness sector and at the same time significantly reduce the regional economic activities of Sambas Regency.
The spread of CVPD depends on the presence of the inoculum and its vectors in the land [2][8]. As a result of CVPD disease, the use of pesticides is very intensive in citrus plantations which causes the reduction of *Diaphorina citri* and natural enemies. This study was to determine the effectiveness of the application of PTKJS and the abundance and diversity of insects.

2. **Methods**

The research was conducted in the gardens of Dul, Syamsudin and Hermanto with an average planting area of 0.5 - 1 ha in Tebing Batu village, Sebawi District, Sambas Regency from February to October 2018.

![Figure 1. Map of location for the demonstration plot of Tebing Batu Village activities, Sebawi District, Sambas Regency](image)

2.1. The methodology of implementing activities is by taking insect samples

Research on diversity, abundance, population dynamics and symptoms of disease attacks that arise in plants or fruit was carried out by survey / observation / monitoring methods of *D. citri* vector CVPD insects and other insects [4][5].

Experiments are compiled based on the application of several observation / monitoring techniques for vector insects and disease symptoms that arise including monitoring technique based on census of all plants showing symptoms (CVPD visual observation method) and the presence of vector insects (*Diaphorina citri*). This monitoring technique is used by observing directly all parts of the plant that show symptoms of CVPD either sectorally or as a whole.

The percentage of attacks is calculated based on the formula:

\[
\text{Percentage of attacks} = \frac{\text{number of plants showing sectoral symptom}}{\text{the total crop in the entire demonstration plot}} \times 100\%
\]

The data obtained were used as a standard for other treatments such as monitoring technique based on the observation of CVPD attacks on 100 samples at harvest, monitoring techniques based on the installation of traps / trapping (*yellow trap*) and trapping with chemical compounds / pheromones (sticky pitfall trap / feromone trap), monitoring technique based on PCR test at the Bacteriology Laboratory of IPB.

2.2. **Implementation of Integrated Management of Healthy Citrus Gardens (PTKJS)**
The application of PTKJS technology to support increased production and quality of citrus fruit that has been applied includes pruning, maintenance, fertilization, irrigation, coating and spraying. Pruning and maintenance is done by pruning unproductive branches, twigs and wild shoots. Before fertilizing, weeding is done, especially those that grow on the reefs. Application of dolomite lime to increase soil pH is carried out in conjunction with chemical fertilizers. The soil in the research location is a soil with a high enough acidity level so that to increase the level of nutrient availability in the soil, the soil acidity needs to be reduced. Fertilization is carried out based on the SOP for citrus cultivation for plants over 5 years, namely by providing organic and inorganic fertilizers in the form of NPK, Urea, and KCl, namely 25 kg of manure / tree, NPK 15-15-15: 375 grams, urea: 867 grams and KCl: 406 grams per plant applied 3 times and Dolomite given 1 kg per tree. Meanwhile, vector control activities were carried out by covering and spraying a systemic insecticide with 200 SL and 50 EC imidacloprid on existing citrus plants. This is done 1 month before further observation activities. This coating and spraying is carried out to reduce the population of *D. citri* that already exists in the citrus plantation environment.

2.3. The criteria for the effectiveness of the CVPD monitoring technique

The criteria for the effectiveness of the CVPD monitoring technique are determined based on the accuracy of the observation method is seen from the percentage of monitoring results against standard methods, the test used to measure the accuracy is the Distribution Equality Test. It also based on the speed of observation is measured based on the work performance of the observer who uses alternative methods on a certain area.

Estimation and significance of vector insect presence (*D. citri*) between the treatment demonstration plots using the T-test at a 95% confidence interval.

3. Results and Discussion

3.1. Identification of the extent of attack and the intensity of CVPD attacks

The research "Development of CVPD (Citrus Vein Phloem Degeneration) Vector Insect Monitoring Techniques and CVPD Symptoms in Pontianak Citrus Plants in an Effort to Suppress Harvest Loss" begins with the determination of the research location. From the results of the location survey, it was found that 3 citrus orchards owned by farmers included Dul, Syamsudin and Hermanto's gardens with an average planting area of 0.5 - 1 ha. All demonstration plots have a plant population between 200-400 trees with a plant age span of more than 5 years, which are located in Tebing Batu Village, Sebawi District, Sambas Regency. Followed by regular sampling of samples during the two harvest periods, all samples were analyzed in the West Kalimantan AIAT Laboratory and the IPB Bacteriology Laboratory.

The initial verification and identification of CVPD-infected plants in all demonstration plots was carried out with the aim of knowing the extent of attack and the intensity of CVPD attacks before the application of the technology package in the form of various monitoring techniques and the application of recommended technology in the form of Integrated Management of Healthy Citrus Areas (PTKJS). Observation of attack intensity is divided into 6 levels of categories, namely: a) 0% (not attacked), b) attacked by 1-20%, c) attacked by 21-40%, d) attacked by 40-60%, e) attacked 61-80%, and f) 81-100% attacked. Plants with an attack intensity of > 60% are recommended to be dismantled and replaced with healthy seeds labeled free from disease, while the intensity of the attack is <60%, for which sectorally weeding and pruning are carried out on branches that are symptomatic of CVPD infection. The results of verification of the intensity of CVPD attacks are in Figure 2 (intensity of CVPD attacks).
Figure 2. Percentage graph of the number of plants attacked based on CVPD attack rate

From the verification results before the CVPD control technology was applied to the treatment demonstration plot which was carried out serially with the total population taken in stages (10%, 20% and 30%) of the total population, it was seen that there was a stable average CVPD attack rate on the land. Meanwhile, the results of observations on the presence of hippopotamus imago (*Diaphorina citri*) as a vector for spreading CVPD in each farmer's land vary widely, in this case it strengthens us that the symptoms that appear in the crop are more to the symptoms of CVPD attacks. Meanwhile, to eliminate bias due to deficiency of micro nutrients such as Mn, Fe, Cu, Br, especially Zn deficiency or by other factors that show symptoms similar to those caused by CVPD, it can be corrected by applying fertilizers.

From the graph, it can be seen that the CVPD infestation already exists in the land with various percentage levels of spread. The largest percentage was in demonstration plot 1 (Dul) with CVPD attack rate category C (21-40%) of 51.33%, while demonstration plot 2 (Syamsudin) and 3 (Hermanto) were at category B CVPD attack rate (1-20%) amounting to 45-50% of the total crop.

If it is related to crop yields, the results of this verification are also highly correlated / affect the volume and grid of citrus fruit harvested, where it is very visible the initial harvest before the implementation of PTKJS and the second harvest after the implementation of PTKJS and monitoring techniques show changes in yields, listed in Figure 3. The graph shows an increase in crop yields between 2.99% in Syamsudin cropping to the highest 24.09% in Dul cropping. Meanwhile, Hermanto's land experienced an increase in yields of up to 18.16% after the implementation of monitoring techniques followed by PTKJS treatment.
Abundance and Diversity of Insects

The results of further identification carried out at the BPTP West Kalimantan integrated laboratory using yellow traps and steiner model traps, from the results of research that has been carried out in 2 harvest periods obtained a total of 8617 individuals. The number of insects was obtained from the three observation demonstration plots over a period of 10 months (February - October 2018) for 10 repeated observations after the implementation of PTKJS. Diversity Types of insect species when grouped into order levels are dominated by the Hymenoptera Order with 17 families from a total of 7515 trapped insects, the Diptera Order with 5 families of 377 insects, the Coleopteran Order with 1 family of 282 insects and the Hemiptera Order with 4 family levels of 140 insects trapped. Meanwhile, the orders of Lepidoptera, Orthoptera and Odonata were not too dominant, further listed in Figure 4.

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Figure 4. Graph of insect species diversity at the order and family level [1][7]
Family *Psyllidae* from the Order Hemiptera with the species *Diaphorina citri* (*D. citri*), especially for the adult level, show very fluctuating population dynamics in the application of several monitoring techniques, further listed in Figure 5.

![Figure 5. Population dynamics of adult *Diaphorina citri*.](image)

In general, during the ten initial observations with various methods of monitoring techniques, it was seen that the total catch of the adult population of *D. citri* between treatments experienced clear and varied changes (very fluctuating). The factors that determine the high and low population of an organism in an ecosystem are very much determined by 3 factors, namely internal factors (required life cycle time, sex and meridian ratios), external factors (the influence of biotic and abiotic environments) and factors the amount of food [6]. To monitor at this time, external weather factors (rainfall, temperature and temperature) that do not determine the location are more determinants and limiting factors for research on the presence / presence of insects in the field and are seen in the low presence level of *D. citri* both before application and implementation of recommended technology (PTKJS).

3.3. The application of the test is based on the presence of vector insects in the field

The monitoring technique for the presence of vector insects in plants with CVPD symptoms in the field at the observation level of 10%, 20% and 30% of the total plants in the treatment demonstration plot before and after the application of PTKJS resulted in high accuracy with the lowest value and was not significantly different from the results. Census on other plants

the data analysis, it shows that the score obtained from the application of this monitoring technique has an average emphasis from 16.33 to 1.67. While the correlation between the catch ability of *D. citri* before and after the application of PTKJS was 0.945 so that there was a significant relationship between the accuracy of visual observations and the presence of *D. citri* before and after the implementation of PTKJS.

The next output is the result of the analysis of the average difference, it can be seen that the mean is 14.667 with a standard deviation of 2.517 with a t count of 10.094, while the Sig (2-tailed) value is 0.010 <0.05, so it can be concluded that H0 is not accepted, so it can be said that there is a significant difference between the catches of *D. citri* before or after the implementation of PTKJS. Likewise, the abundance of eggs and nymphs of *D. citri*, can be seen in Figure 6.
3.4. Results of the application of various test monitoring methods.
From the results of the study, it is known that the method with the Polymerase Chain Reaction (PCR) test at the Bacteriology Laboratory of IPB from plant parts (leaves) obtained from the research demonstration plot in the field that shows the symptoms of sectoral blotching can indicate a real CVPD attack. Thus, this method can be used as an indicator / standard research method because it has high accuracy. Polymerase Chain Reaction (PCR) test is a molecular method that can amplify DNA sequences exponentially [9]. The PCR method is a very sensitive method and can be used to detect pathogenic bacteria that cause CVPD disease even though their concentrations are very low in plant tissue [10][11]. The pathogenic bacteria that cause CVPD are obligate so that they cannot be cultured in artificial media, therefore the DNA template used in PCR is obtained from the results of the total DNA extraction of lime leaf samples. The specificity and sensitivity of the PCR method with primers used in this study has been well tested to amplify 16SrDNA from the Liberobacter asiaticum, (L. asiaticum) strain.

![Figure 6. Percentage of abundance of Diaphorina citri, nymph and adult levels.](image)

![Figure 7. Results of 16SrDNA amplification from L. asiaticum strains](image)

Detection of the results of total DNA isolation from symptomatic / sick leaf samples showed the presence of DNA bands at 1160 bp of 1% agarose gel electrophoresis. Whereas in healthy /
asymptomatic samples, no DNA bands were seen at 1160 bp, it can be seen in Figure 7. The percentage of CVPD attacks on each monitoring method is then compared with the results of the standard method CVPD attack (PCR) census to determine the level of monitoring accuracy. The monitoring technique based on visual symptoms in plants that show symptoms of sectoral blotching followed by PCR test has the highest accuracy as evidenced by testing 27 samples repeated 3 times showing >90% positive indicating the presence of *L. asiaticum* bacterial infection that causes CVPD, shown in Figure 11. This is indicated by the 27 samples tested >90% of the samples showed the presence of DNA bands at 1160 bp and only under 10% did not show any DNA band formation. With the formation of DNA bands at 1160 bp, this indicates that the leaf sample is positive for *L. asiaticum* bacteria, because the presence of these bacteria is detected by 16SrDNA primer which is the sequence that must be present.

4. Conclusion
Population dynamics of *Diaphorina citri* are influenced by the high and low population levels in an ecosystem, the length of the life cycle, the influence of biotic and abiotic environments, food availability factors, and natural enemies. The implementation of monitoring techniques that were continued by PTKJS, there was the highest increase in crop yields on Dul's land of 24.09%, 2.99% in Syamsudin land. Meanwhile, Hermanto's land has increased yields of up to 18.16%. The highest diversity of insect species trapped after the implementation of PTKJS was dominated by the Hymenoptera Order with 17 families out of a total of 7515 trapped insects. The application of this monitoring technique has an average emphasis on *Diaphorina citri* from 16.33 to 1.67. The PCR method is a very sensitive method and can be used to detect pathogenic bacteria that cause CVPD based on testing of 27 samples repeated 3 times showing >90% positive indicating the presence of *L. asiaticum* bacterial infection that causes CVPD.

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