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Research Article
Evaluation of Acid-Based Pheromone for Monitoring *Diaphorina citri*, Vector of Huanglongbing Diseases Under Tropical Climate

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Abstract
Background and Objective: *Diaphorina citri*, also called the Asian citrus psyllid (ACP), is a vector for huanglongbing (HLB) disease. It has been identified as a significant problem for citrus farmers worldwide. However, no systematic monitoring programs specifically target ACP populations in the tropics, such as Indonesia. This study tests the efficiency of yellow sticky traps with acid-based attractants, such as acetic (AA), formic (FA) and propionic (PA) acids. **Materials and Methods:** The concentration of each acid was 1 µL acetic acid (AA), 0.25 µL formic acid (FA) and 0.1 µL propionic acid (PA). The study was conducted in three different controlled conditions: Semi-field and open field, with low and high population scenarios. The data obtained were subjected to analysis using a One-way Analysis of Variance (ANOVA) with a confidence level of 95% and differences were analyzed using Tukey's Honestly Significant Difference (HSD) test. **Results:** In the under-controlled setting, AA was the most effective, with 13 adult ACP captures, which is 4.3 times more than the control (3.00). In the semi-field condition, PA had the best result of 3.8, which is 1.4 times higher than the control (2.6). In the low population scenario of the open field condition, AS had the highest ACP catch of 4.1, which is 17.1 times higher than the control (2.33). On the other hand, the high-population AA condition had the highest result of 13.71 or 4.1 times higher than the control (3.29). **Conclusion:** The utilization of acid attractants, whether AA, FA or PA, is effective in increasing the catch of adult ACP under tropical conditions. However, the performance of the three types of acids was not stable in the three series of studies that were conducted.

Key words: Acetic acid, *Diaphorina citri*, formic acid, huanglongbing, monitoring, propionic acid

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The vector of huanglongbing, *Diaphorina citri* or Asian citrus psyllid (ACP), is still at the top of the list of pests that significantly affect citrus production[1-3]. Almost all citrus-producing countries have high concentrations of this invasive pest, *D. citri* and Indonesia is no exception[4-6]. Various efforts have been made to tackle this pest, including finding the right method to monitor ACP populations[7]. Pheromones are a reliable option for monitoring ACP in citrus fields. Various pheromones have been developed and used for ACP control, including acidic ones[8]. The utilization of this acid is beneficial for the citrus industry across many countries, given the paucity of manufacturers specializing in producing pheromones or traps explicitly designed to combat the Asian citrus psyllid. Pheromone-based is the primary key to attracting ACP, especially males; Luo *et al.*[9] the study provides information that male ACPs are strongly attracted by acetic acid doses of 0.1-10 µL. Sticky traps remain critical to monitoring and managing ACP pests[10,11]. Incorporating pheromone lures has been shown to enhance the efficacy of trapping, particularly in the instance of acid-based pheromones[9].

Extensive research has confirmed that tropical regions have higher humidity and temperature levels than other regions. Tropical regions, notably Indonesia, provide optimal conditions for ACP development[12]. As the largest citrus producer in Southeast Asia, Indonesia is a vital subject for research on Asian citrus psyllid (ACP) and huanglongbing (HLB) disease[13]. The distribution of the Asian citrus psyllid (ACP) and huanglongbing (HLB) is significantly influenced by environmental factors[14], particularly temperature and humidity[15].

In the Indonesian context, the ACP represents a significant challenge for citrus plantations[6,16], with the pest identified as a national control priority. Currently, the cultivation of citrus fruits is practiced in various locations across Indonesia, including the islands of Bali, Java, Sumatra, Kalimantan and Sulawesi, with differing production levels in each area[17]. The ACP is a significant concern for farmers in the island's citrus region[7], which is produced not only in Indonesia but also in almost all citrus countries. It is of particular interest to note that, at the presently available level of knowledge, there is no practical method for dealing with ACP pests in citrus crops[18]. This renders the utilization of pheromones a pivotal prospective avenue of investigation. Investigating ACP trapping with pheromone acids in tropical environments is crucial and will contribute significantly to regional pest management. This study aimed to assess the efficacy of an acid-based pheromone for monitoring populations of *D. citri*, the vector of huanglongbing disease, under tropical climate conditions.

MATERIALS AND METHODS

Study area: This research was conducted from November, 2023 to the end of February, 2024. A series of experiments were conducted under controlled conditions at the Faculty of Agriculture research facility at Universitas Gadjah Mada. Semi-field treatments research was conducted at Experimental Farming, Purworejo Regency, Central Java Province. Meanwhile, field trial research was conducted in Bengkulu City, Bengkulu Province, Indonesia.

Acid-based pheromone preparation: Three acidic compounds were evaluated as potential treatments: Acetic acid, formic acid and propionic acid. Additionally, commercial pheromone products from Alpha Scents Inc., were included for comparison. The acid concentrations in these mixtures were based on the concentrations that have been previously demonstrated to effectively elicit ACP catches[8,19]. The concentration of each acid was 1 µL acetic acid (AA), 0.25 µL formic acid (FA) and 0.1 µL propionic acid (PA). The optimal concentration was determined by Zanardi *et al.*[8] and represents the most optimal value. As recommended, the solution was prepared by taking one microliter of acetic acid (AA) and dissolving it in 1 µL of hexane. Similarly, 0.25 µL of formic acid (FA) solution was prepared by mixing 0.25 µL of formic acid with 0.75 µL of hexane, shaken evenly, before use in laboratory and field tests. A 0.1 µL PA solution was prepared by mixing 0.1 µL of PA with 0.9 µL of hexane, as with the other acids. Each solution comprising the acids acetic (AA), formic (FA) and propionic (PA) was prepared in a volume of approximately 15 mL as a primary stock solution and stored in a refrigerator at a temperature of $\pm 18^\circ$C until required for analysis.

Pheromone packages: In the implementation of the study, handmade pheromones with pre-made formulas (either AA, FA or PA) were placed in 0.5 mL tubes during the study. Previously, the tube was perforated with a needle, creating a 1 mm hole sufficient for the pheromone to be exposed. A total of 0.3 mL of stock pheromone solution (AA, FA and PA) was applied over the seven days treatment period. The 0.3 mL quantity was determined through preliminary trials, ensuring that it would be sufficient for the entire treatment duration. During operation, the conduit is fastened in the central position on the card using a nylon thread tie, with the aperture positioned upwards. Given the lack of synergy exhibited by the acids, namely AA, FA and PA[8], a sequential approach is employed to optimize the efficacy of the trapping process.

Evaluation under controlled conditions: The treatment of controlled substances was conducted in cages inside the greenhouse. A cage with dimensions of 1×1×1.2 m (length×width×height) was employed as a treatment. The cage was enveloped in a 50 mesh net, equivalent to a 0.3 mm mesh size. The treatment utilized a cohort of approximately one year old citrus plants positioned in the center of the cage. Yellow sticky trap (YST) cards from Alpha Scents (Alpha Scents Inc.) were affixed to the exterior of the citrus canopy at an approximate height of 0.75 m from the surface. The treatment was conducted for seven days, after which the number of ACP caught on the cards was observed and recorded. This study employed a completely randomized design with five treatments (AS, AA, FA, PA and control) and four replicates per treatment. This study utilized a controlled release method, introducing 30 adult ACP (4-6 days old) per citrus tree. A 2 hrs acclimation period allowed ACP to migrate from transfer containers to citrus foliage before installing YST and pheromone lures. The citrus used were 1.5 year old citrus siamese (*Citrus nobilis*) with new flush, cultivated in 35 cm polybags. The research site for controlled conditions is in the research facility of the Faculty of Agriculture of the University of Gadjah Mada.

Evaluation of semi-field treatments: A semi-field treatment is a cultivation method that utilizes citrus plants in open-field settings, with a screen house providing protection. In this instance, the screen house has dimensions of 6×12 m^2 and houses 15 citrus plants. The distance between plants is maintained at 1.5×1.5 m by the proportional planting scheme. In this study, "oranges" refers to a specific type of citrus fruit, the Siamese orange (*Citrus nobilis*), which has been allowed to reach one year's maturity after planting.

In this treatment, 30 freshly emerged adult ACPs were released to each citrus plant in the greenhouse. Following this, a 2 hrs waiting period was observed to allow the ACP to settle on the plants before treatment with YST and pheromone cards was initiated. At a height of 1.5 m above the surface, YST cards are suspended from the outermost canopy. This semi-field treatment was left for seven days and replaced with a different type of pheromone. Each treatment was repeated ten times. Semi-field treatments were conducted at Purworejo Experimental Farm (7°42'43.0"S, 109°55'56.1"E) in Central Java, Indonesia, during January and February, 2024.

Evaluation of field trial: In agricultural practice in the Bengkulu region, the field trial constitutes a treatment method deployed in citrus-cultivating fields. The treatments were conducted in two distinct scenarios. Two distinct scenarios were employed: One comprising a high population and the other a low population. The high population scenario was established by allowing the natural population conditions to remain high in the open farmland. Conversely, the low population was established by enclosing the selected plants within a 2 m high net.

The duration of the field trial treatment was identical to that of the cage and semi-trial, which lasted seven days. Six replications were conducted in both the high and low population scenarios.

The trial was executed in an agricultural research center located in Padang Serai, Bengkulu City (3°54'16.1"S, 102°19'18.2"E), situated within the province of Bengkulu, from January to February, 2024.

Environmental data collection and data analysis: The environmental parameters recorded during treatment comprised temperature and humidity, with these data collected via an Elitech RC-4HC data logger (Elitech.com log version 7.2.1) at 10 min intervals throughout the study. This approach provided a detailed representation of local environmental conditions and the collected data were verified in Excel files (Microsoft Inc.) to ensure accuracy. Subsequently, the data were visualized in graphical form for more straightforward interpretation. The tabulated data set, including all ACP capture data from the various treatments, was subsequently analyzed using Analysis of Variance (ANOVA) to ascertain significant differences. Tukey's Honest Significant Difference (HSD) test was also employed to identify specific differences between the pheromone treatment groups. Statistical analysis was performed using IBM SPSS Statistics software, version 25.0. One-way Analysis of Variance (ANOVA) was employed to evaluate significant differences in ACP capture rates among pheromone treatments. Subsequent *post hoc* Tukey's HSD tests were conducted to ascertain the pairwise differences between treatment groups.

RESULTS

Acid-base lures performance under controlled conditions: A controlled environment assessment is required to independently validate the efficacy of homemade and commercially manufactured pheromone lures under defined conditions. The YST results show different ACP responses to the lures given in Table 1. The best performance of YST catches is acetic acid (AA). However, this needs to be tested with ANOVA. The results of ANOVA analysis showed that there was a significant difference between treatments (Table 1), namely the provision of acetic acid (AA), Formic Acid (FA), propionic acid and alpha scents (AS) commercial lures. This encourages Tukey's HSD test to find out how and where the difference occurs, so that it can provide further information. A study was conducted to ascertain the efficacy of various contraceptive methods on adult ACPs. The results indicated that AA was found to be a more efficacious method compared to FA (9.25±2.59), PA (7.25±3.27), AS (6.25±2.86) and control (3±1.87). The mean ACP catch for AA was recorded as 13±5.39, significantly more than the mean values for the other methods.

Tukey's HSD results showed that the control treatment (without using lures) significantly differed from the other treatments, except with AS (commercial lures). Meanwhile, Tukey's HSD test results between commercial lures (AS) against three homemade formulas, AA, FA and PA, showed no significant difference (Table 1). Likewise, treatments with artificial lure formulas did not show significant results between treatments. This confirms that the best results of AA for ACP catch in YST were only different from the control (without lures), while the other lure treatments were not different.

The climate plays a pivotal role in assessing the efficacy of lures incorporated into traps. As illustrated in Fig. 1, the temperature and humidity exhibited fluctuations during the experiment, conducted under controlled conditions. The mean temperature range was documented to be 30.08°C, while the mean humidity level was recorded as 72.77%.

Acid-base lures performance under semi-field conditions: Preliminary testing under semi-field conditions facilitates the evaluation of pheromone efficacy, ensuring optimal performance in tropical climates. Semi-field experiments conducted within mesh-covered enclosures optimize the monitoring and retrieval of adult ACP following treatment application. Table 2 illustrates the comparative effectiveness of AS, AA, FA and PA pheromones and control on adult ACP trapping. The present study sought to determine the value of ACP when caught using YST equipped with lures, finding that FA (3.2±2.17) had the highest value of all, followed by PA (3.8±1.30), AS (2.6±1.52), C (2.6±2.70) and AA (2.2±1.30). Table 2 also showed that PA had the highest catch (3.8) of adult ACP caught with YST traps. However, this needs to be confirmed with ANOVA analysis and Tukey's HSD test to determine whether there is a fundamental difference between the various treatments in terms of the number of adult ACP trapped.

The ANOVA results showed no significant difference between the treatments (Table 2). This means that providing various lures in semi-field conditions does not always significantly impact the catch of adult ACP with YST. Statistical analysis shows no significant differences (p>0.05) in ACP capture rates among treatments with homemade (AA, FA and PA), commercial (AS) and without (control) lures.

Table 1: Average number of adult ACP captured in YST with various lure treatments under controlled conditions

Treatment	Average of ACP captured
Control	3.00 ± 1.87^{a}
Alpha scents	6.25 ± 2.86^{ab}
Acetic acid	13.00 ± 5.39^{ab}
Formic acid	9.25 ± 2.59^{ab}
Propionic acid	7.25 ± 3.27^{b}

*Values in the same column followed by different letters differ significantly under Tukey's HSD test (α = 5%)

Table 2: Average number of adult ACP captured in YST with various lure treatments under semi-field conditions

Treatment	Average of ACP captured*
Control	2.6 ± 2.70^{a}
Alpha scents	2.6 ± 1.52^{a}
Acetic acid	2.2 ± 1.30^{a}
Formic acid	3.2 ± 2.17^{a}
Propionic acid	3.8 ± 1.30^{a}

*Values in the same column followed by different letters differ significantly under Tukey's HSD test (α = 5%)

Table 3: Average number of adult ACP captured in YST with various lure treatments under field conditions

	Average of ACP captured	
Treatment	Low population*	High population*
Control	2.33 ± 2.34^{a}	3.29 ± 3.90^{a}
Alpha scents	41.00 ± 34.30^{b}	5.00 ± 4.69^{ab}
Acetic acid	8.83 ± 7.11^{a}	13.71 ± 5.65^{bc}
Formic acid	10.50 ± 8.50^{a}	20.14 ± 9.82^{c}
Propionic acid	10.67 ± 9.24^{a}	6.14 ± 5.30^{ab}

*Values in the same column followed by different letters differ significantly under Tukey's HSD test (α = 5%)

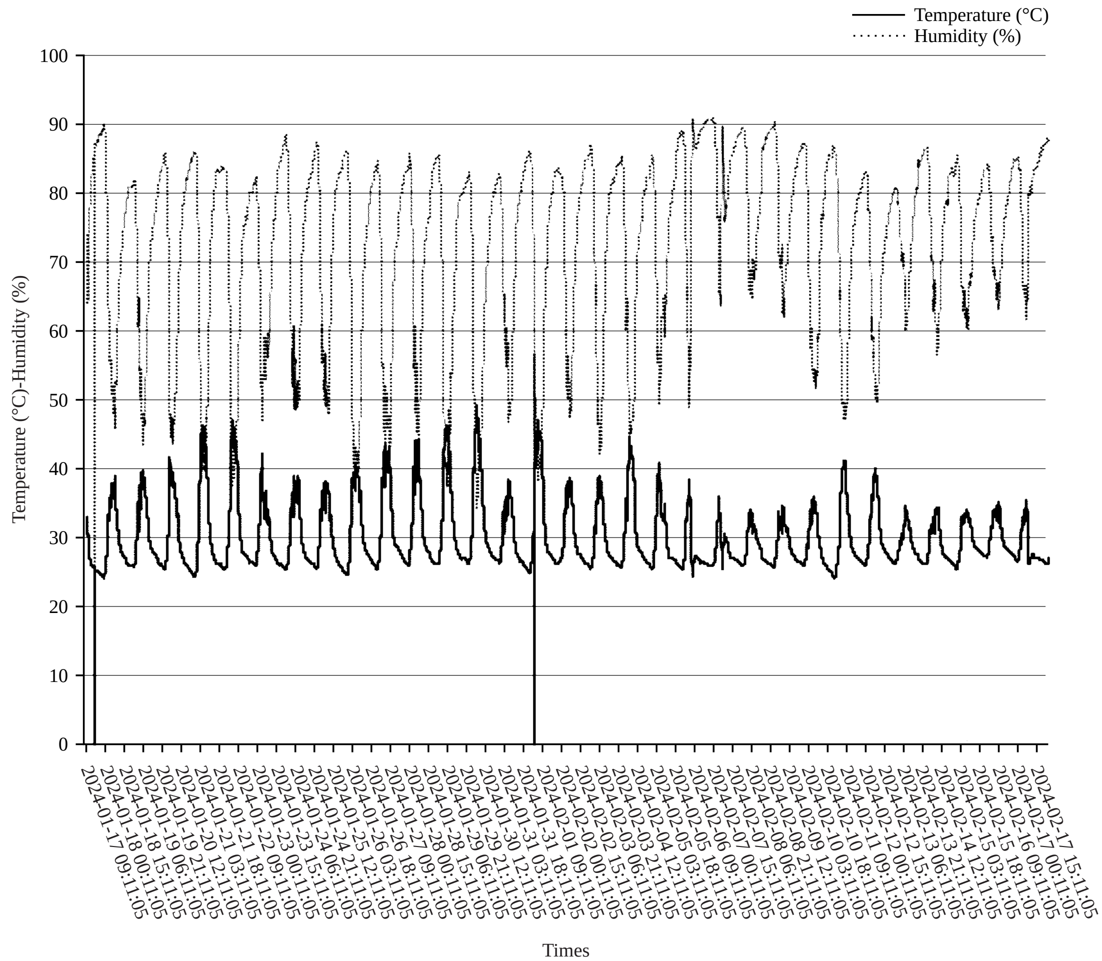

Fig. 1: Temperature (°C) and humidity (%) values recorded during the study in controlled conditions

The environmental conditions, including temperature and humidity, recorded during the experiment are summarized in Fig. 2. The temperature in the Purworejo area was 25.83°C, with an average humidity of 66.73%.

Acid-base lures perform under field conditions: The findings from the low-pressure population environment suggest that the application of AS resulted (Table 3) in the highest catch (41.00±34.30), followed by PA (10.67±9.24), FA (10.50±8.50), AA (8.83±7.11) and the control group (2.33±2.34). This finding shows that applying handmade (AA, FA and PA) or commercial (AS) pheromones can enhance catch rates. However, employing the Tukey's HSD test to discern the differences among the various pheromone treatments is essential.

Tukey's HSD test revealed a significant difference in outcome between AS and the other treatments, namely the other pheromones (homemade) and the control group (Table 3). In contrast, the second group, including homemade and control treatments, exhibited no statistically significant variation in catch data. However, it should be noted that PA appeared to be the most effective treatment, followed by FA, AA and the control group. This outcome is attributed to the presence of SEM value variations.

The findings of the field experiment in which YST was trapped in high-pressure populations are illustrated in Fig. 3. The results prove that under high-density conditions, the optimal outcome was achieved with FA (20.14±9.82), followed by AA (13.71±5.65), PA (6.14±5.3) and AS (5.00±4.69). Conversely, the treatment devoid of pheromone (named the "control") yielded the lowest catch rate compared to the other treatments, namely the homemade and commercial pheromone treatments.

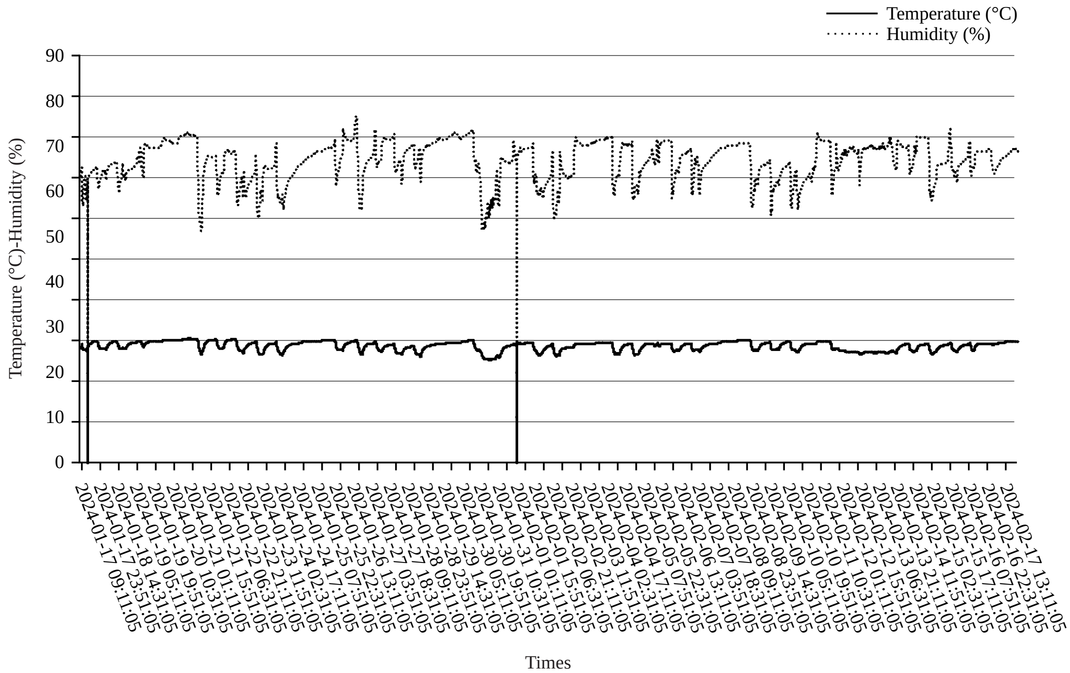

Fig. 2: Temperature (°C) and humidity values (%) recorded during the study under semi-field conditions

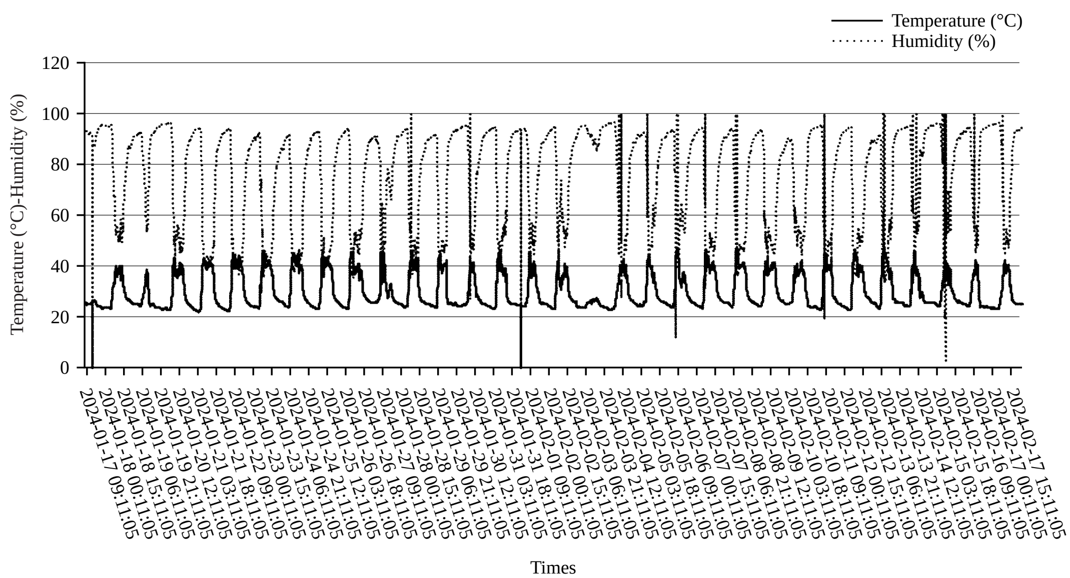

Fig. 3: Temperature (°C) and humidity (%) values recorded during the study under field conditions for both low and high-population scenarios

The Analysis of Variance (ANOVA) of the high-pressure population proved that all treatments exhibited a significantly different impact with a confidence level of 95%. This finding suggests that providing lures, whether commercial (AS) or homemade formulas (AA, FA and PA), exerts a substantial influence in capturing adult ACP in high populations within citrus fields. Consequently, further experimentation is necessary to learn the extent of variation among the treatments.

The Tukey's HSD test revealed that under conditions of high population density, three distinct groups (shown by a, b and c) were evident in the response results. The application of PA and AS enhanced the catch of ACP beyond that observed in the absence of pheromone (control). Conversely, AA exhibited the highest catch compared to AS and PA. Finally, FA yielded the highest catch (20.14±9.82), significantly different from the control, AS, AA and PA.

The environmental conditions in Bengkulu, which is located near the equator, are presented in Fig. 3. The temperature was elevated, exceeding 30°C and the humidity was relatively stable, ranging from 70 to 80% at almost all times.

DISCUSSION

The outcomes of the three series showed that the formulation of both homemade (AA, FA and PA) and commercial (AS) acids resulted in enhanced adult ACP capture through trapping protocols (YST). However, the three-research series showed that the three pheromone acids, irrespective of their homemade or commercial origin, exhibited varied optimal performance in achieving maximum catch yields. The observed increase in the number of catches following the addition of these acid-based pheromones (AA, FA, PA and AS) is consistent with the findings reported by Zanardi *et al.*[8]. Furthermore, the findings of George *et al.*[20] and George *et al.*[21] align with the observations regarding the efficacy of AA and FA in trapping adult ACP. AA and FA trigger the catch increase through the host selection mechanism.

In the three distinct series of experiments produced, AA exhibited the highest levels of performance (Table 1) in a controlled environment. At the same time, PA demonstrated the most excellent efficacy in semi-field conditions (Table 2). Conversely, AS yielded optimal outcomes in low-population scenarios and FA in high-population scenarios in the open field (Table 3); it is understood that AA is an active compound that can attract male adult ACP [9,19,22] under various conditions. In contrast, FA and PA have been demonstrated to possess remarkable electroantennographic (AEG) properties, particularly in adult ACP[21]. The inconsistency of each attractant, as indicated in the three distinct research series (controlled, semi-field and open field), can be caused by the absence of data concerning the proportion of adult ACP males and females. However, under specific conditions, the female population can exceed the male population[23].

The results of the YST trapping series in controlled areas, semi-fields and open fields with lures of AS, AC, FA and PA indicated different results. Propionic acid-based lures effectively increased trapping in controlled areas but were less effective with the AS in semi-field areas. This result agree with Zanardi *et al.*[8] that PA is more effective in catching adult ACP than FA and AA, so it also represents PA as the best choice to trap ACP in the field. The three chemical components used, AA, FA and PA, are part of the sex pheromone of ACP, which is generally used for communication[19,21,23]. The response of ACP YST trapping from a series of experiments showed an increasing trapped adult ACP compared to the control (without lure). These results are promising in developing either composition, combination or other formulations of lures that more effectively control/monitor ACP. In a low population (N = 30 adult ACP), the best performance of lures for monitoring ACP is shown. This result indicates that in tropical climates, including Indonesia, propionic acid has a better chance of being selected as the best lure to control or monitor ACP in the field. These results are somewhat different from another study by Luo *et al.*[9], which shows acetic acid as the best potential lure for ACP monitoring.

The life cycle of ACP, extending from the egg to the adult stage, is significantly influenced by environmental factors such as temperature and precipitation[24]. During the specified study period, environmental variables, including temperature and humidity, were measured and the results indicated minimal discrepancies among the three study series. Furthermore, the semi-field study conducted at Purworejo (Fig. 2) and the open-field study conducted in Bengkulu (Fig. 3) showed the actual environmental conditions of the local area, thereby validating the experimental setting and underscoring the relevance of the research findings to their original context. As illustrated in Fig. 2, Purworejo (7°42'43.0"S, 109°55'56.1"E), consistent with other regions along the Southern Coast of Java Island, exhibits a mean temperature below 30°C during January through March. Bengkulu (3°54'16.1"S, 102°19'18.2"E), being situated closer to the equator, shows significantly higher temperatures and humidity levels (Fig. 3) during the experiment in comparison to Purworejo. This pattern is subject to disruption only in extreme weather events, such as El Niño[25,26], during the study period. In the 2023-2024 period, the dry season typically extends, accompanied by a decline in precipitation. The population dynamics of ACPs in natural habitats are influenced by various ecological factors, including host preferences[21], the presence of buds[3,27], thermal requirements[28] and responses to visual and chemical volatile stimuli[29,30]. The prolonged dry period (in the case of El Nino) has been shown to reduce the rate of citrus buds' development[31]. This phenomenon naturally exerts downward pressure on the ACP population.

CONCLUSION

The incorporation of attractants composed of acetic acid, formic acid and propionic acid into YST has been demonstrated to result in a substantial enhancement of the capture efficiency of adult ACP. Inconsistent efficacy was observed in the three pheromone acids under various conditions, including controlled, semi-field and field experiment settings. The commercial attractant (AS) emerged as the most effective option within a low-population scenario. In contrast, in high population conditions, formic acid (FA) was the most efficacious in augmenting the catch of ACP. An in-depth investigation is warranted, explicitly focusing on low ACP population scenarios, to inform effective pest mitigation and dissemination prevention strategies.

SIGNIFICANCE STATEMENT

This study identified acid-based attractants, particularly acetic acid and propionic acid, as effective agents for enhancing the capture rate of *Diaphorina citri*, which could be beneficial for monitoring and managing vectors of huanglongbing disease in citrus orchards. This study will assist researchers in uncovering critical areas of vector behavior and attractant efficacy that have remained unexplored by many. Consequently, a new theory on attractant-based integrated pest management strategies may be developed.

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