Nuisance Insects of Rearing mass Asian Citrus Psyllid (ACP) *Diaphorina citri* Under Controlled Conditions

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Abstract. Rearing insects for pest experiment it's very critical. The condition of rearing such insects impacted the smooth running of research. This condition also happens in the rearing of Asian Citrus Psyllid (ACP), *Diaphorina citri*. ACP is one of the most dangerous pests of citrus since responsible for the huanglongbing disease vector in citrus. Many researchers, either laboratory or field-based use a *D.citri* for object experiments. A clear and recent description of the types of insects or arthropods that disturb the rearing ACP is needed, especially in Indonesia. The research was conducted in the greenhouse of Faculty of Agriculture, Gadjah Mada University, from January 2021 to September 2022. The rearing of ACP uses orange jasmine (*Muraya paniculata*) and citrus (*Citrus nobilis*). The collected insect and spider preserved in alcohol or pined in insect tray and identify them based on related literature. This study proposed to assess the nuisance insect that infested on rearing of D.citri based on greenhouse conditions. Our results showing that the many nuisance insects that disturb the growth of rearing in *D.citri.* A total of nine insect and spider attacking *D citri*, they are black ants (*Dolichoderus* sp), aphid (*Aphis* sp), leaf roller caterpillar (*Phyllocnistis citrella*), cockroach (*Blattella* sp), ladybug (*Exochomus nigromaculatus*), mite (*Tetranychus sp*), Mealy bug (*Pseudoccocus* sp), Scale insect (*Aonidella aurantia*) and Spider. Those nuisance insects and spiders attack *D.citri* colonies in different ways and stages. The attacking nuisance insect affected to affected the size of the ACP culture.

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1 Introduction

Rearing of any insects is important for providing insects in any level of generation for specific research interests. Rearing main purpose is to provide reliable and affordable high-quality insects (Leppla, 2009), for sure it's dependent on researcher needs. Rearing of insect including ACP, either mass or laboratory scale is key roles for successful of any insect treatment. Researchers or users need to pay attention to insect rearing since some effects on insects from laboratory adaptation, inbreeding depression, inadvertent selection, or direct rearing effects (Sørensen et al., 2012). Since rearing insects is critical to any scope of insect research, the rearing condition becomes very important.

Diaphorina citri (DC) or known as Asian Citrus Psyllids (ACP) are one of the most responsible vectors, transmitting Huanglongbing disease in almost every country around the world (Berk, 2016; Urbaneja et al., 2020). Rearing *D.citri* has been recently very important for studies of the transmission of bacteria causing hualongbing, *Candidatus Liberibacter asiaticus* (Las) (Li et al., 2006; Paris et al., 2013). Providing good and healthy D.citri during the experiment are challenging since any nuisance insect or other organisms bothering *D.citri* growth. The nuisance insect recorded in disturbing *D.citri* growth and colony such as *Tamarixia radiata* (Irvin et al., 2021; Kalile et al., 2022), coccinellids (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), syrphids (Diptera: Syrphidae) and spiders (Arachnida: Araneae), and *Diaphorencyrtus aligarhensis* Shafee, Alam & Argarwal (Corallo et al., 2021; Michaud, 2004). The extensive method of rearing ACP described by (Skelley & Hoy, 2004) includes prerequisites of their parasitoids.

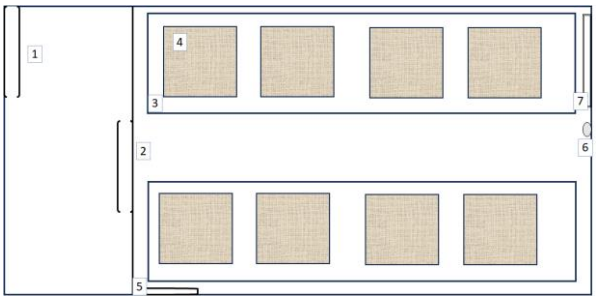
2 Materials and method

2.1 Rearing set

The rearing of ACP uses two common plants, orange jasmine (*Muraya paniculata*) and Sweet orange (*Citrus nobilis*). The rearing is set in a 1x1x1 meter square cage covered with net cloth, the cage maintained under a greenhouse with 27±1 °C and 60% Humidity. Every cage contains six to eight individual plants depending on their canopy. The plant was pruned two weeks before use as a feed for ACP. Every month plants are fertilized with common NPK fertilizer through diluted two spoons (equal to 2 grams) of fertilizer in 10 liter fresh water and poured to plant media as needed. The designs of the greenhouse and rearing set are described in Fig 1. Greenhouses typically consist of two separate doors (no.1 and 2) to prevent insects and other unwanted organisms from entering the rearing area. Inside of green house consisted two benches separately (no. 3) and four cages (1X1X1m) (no. 4) on the bench. The greenhouse is also provided with a fan (no.7) and humidifier apparatus (no. 5) to maintain temperature and humidity under optimal conditions for ACP rearing. Meanwhile, for watering, a water tap equipped with a hose is provided (no. 6)

2.2 *D.citri* infestation and nuisance insect observation

Ten to fifteen of matured adults ACP laid on new shoots of *M.paniculata* and *C.nobilis* using an aspirator. The colony was covered with a 10 cm diameter of a plastic tube covered with net cloth. The colony was left inside the tube for six days and opened immediately after the egg was laid. Eggs are allowed to hatch naturally and are monitored regularly. During the development of egg *D.citri*, observations are conducted at least every week to see if nuisance insects are coming and disturbing. The individuals of nuisance insects are collected and noted if available. The collected insect and arthropod are preserved in alcohol or pinned depend on their size and type of body. All the collected insect and and arthropod are identify based on relevant literature and online catalogue such as Encyclopedia of Life (https://eol.org/).



Note: 1) First door; 2) Second door; 3) Bench; 4) cages (1x1x1m); 5) Humidifier; 6) Water tap; 7) Fan

Fig. 1. Design of Greenhouse and rearing set during observations of nuisance insect.

3 Results and discussion

The results during observation rearing facilities of ACP are provided in Table 1. Every insect and arthropod have different type of disturbing even predator, parasitoid, or competitor. Those roles are describing as possible their type of disturbing and stadia of ACP.

Table 1. Kind of nuisance insect and arthropod in rearing facilities of ACP.

No	Organism	Possible role	Symptom appearance	Stadia disturbed
1	Black ants (*Dolichoderus* sp)	Predator	Unknown	Egg and nymph
2	Aphid (*Aphis* sp)	Competitor	Leaf curling, especially	Egg and nymph

			in new flush	
3	Leaf roller caterpillar (*Phyllocnistis citrella*)	Competitor	The leaves roll up and stick to each other	Egg and nymph
4	Cockroach (*Blattella* sp)	Predator	Unknown	Egg, Nymph, and imago
5	Lady bug (*Exochomus nigromaculatus*)	Competitor, Predator	pale spots on leaves	Nymph and imago
6	Mite (*Tetranychus* sp),	Competitor, Predator	solid white spots on the leaf surface	Egg, Nymph, and imago
7	Mealy bug (*Pseudoccocus* sp),	Competitor	yellow spot and adherent white colonies on leave and stem	Egg, Nymph, and imago
8	Scale insect (*Aonidella aurantia*)	Competitor	The leaves turn white and the stems dry white	Egg, Nymph, and Imago
9	Spider	Predator	marked by the emergence of the web	Egg, Nymph, and imago

Some insects are commonly pests in the rearing facilities of ACP in every country. Skelley & Hoy (2004) described them very well of Scale insects and Aphid as quarantine pests who mostly attack ACP rearing mass. In our greenhouse and rearing facility's Scale insects, *Aonidella aurantia,* and Aphid, *Aphis sp* are two common pests who disturb the rearing colony in almost every season. The possibilities of Scale insects and aphids coming from the unclear plant, *Muraya*, and *Citrus*, when they were used as prey for rearing. These problems are common in ACP rearing as (Skelley & Hoy, 2004) mentions in their notes. *Muraya* and *Citrus* are favoured host plants for ACP (Hall, 2014; Setyaningrum et al., 2023) which also naturally favor other insects such as Thrips, *Frankliniella spp* ((Hall, 2014), scale insects (Poudel et al., 2022), and any other insect from various orders (Zuhran et al., 2021).

Spider, especially from the Aranea order is the mostly diverse group, consisting of over 42,000 species in 109 families, which are all predators and primarily feed on insects (Humenik et al., 2011). This situation makes Aranae very easy to find in every place where insects settle. In tropical rearing ACP facilities such as, it's almost found in every season both wet and dry season. It's also happened in nature or citrus orchards such as West Kalimantan (Zuhran et al., 2021). Mites are citrus pests, that have a symptom bronzing or silvering effect on the leaves where they attacked on(KENNETT et al., 1999). Differences from the spider, mites mostly attacked plants rather than *D.citri* colony. In these situations, mites become competitors or specific predators, especially on eggs of D.citri.

The black ant, *Dolichoderus sp* is commonly associated with seasonal fruit trees, including Sapodilla and Citrus (Jutsum et al., 1981; Van Mele & Cuc, 2001). Black ants in some cases came to the citrus trees associated with another insect, such as Scale insect (Jutsum et al., 1981; Michael Githae et al., 2020). These situations may happen in the greenhouse, since we found Scale insects *Aonidella aurantia* and *Dolichoderus* sp, in citrus and Muraya. Controlling Scale insects will impact directly or indirectly to *Dolichoderus* infestation inside rearing facilities. Mostly, the infestation of scale insects comes from the previous stock of plant rearing. As we know, scale insects are one of the serious insects that are difficult to control even when applying pesticides. It could happen also in the Leaf roller caterpillar (*Phyllocnistis citrella*) which is a common pest in citrus (Moore & Duncan, 2017), especially in the dry season (Foda et al., 2021). The leaf roller disturbing rearing since young leaves, which *D.citri* are the suitable places for growth. By infestation leaf roller, *D.citri* is unable to lay eggs. The possibilities of pathway's nuisance insect and arthropod-infested *D.citri* rearing are described in Fig 2.

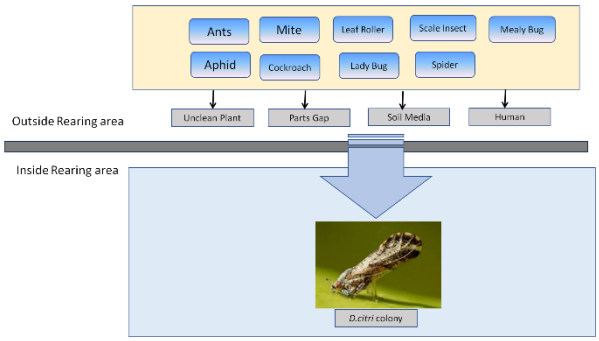


Fig. 2. Nuisance Insect and Arthropod Pathways Disturbance of ACP rearing

4 Conclusion

Nuisance insects of rearing ACP are black ants (*Dolichoderus sp*), aphid (*Aphis sp*), leaf roller caterpillar (Phyllocnistis citrella), cockroach (*Blattella sp*), ladybug (*Exochomus nigromaculatus*), mite

(*Tetranychus sp*), Mealy bug (*Pseudoccocus sp*), Scale insect (*Aonidella aurantia*) and Spider. That nuisance insect played a different role in disturbing the growth of ACP colony. The possible role could be competitor or predator which affected to any stages of ACP growth. The potential pathways that allow nuisance insects and arthropods to pass through unclean plant host sources, gaps in rearing facilities, or through human movement during the rearing process.

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