LIFE HISTORY, HOST PREFERENCE, AND SPATIAL DISTRIBUTION OF *Aphytis chrysomphali* MERCET (HYMENOPTERA: APHELINIDAE) IN THE PHILIPPINES

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ABSTRACT

The life history of *Aphytis chrysomphali*, an ectoparasitoid and one of the common natural enemies of coconut scale insect (CSI), was studied under laboratory conditions using *Aspidiotus destructor* as host. It completed its development from egg to adult in 14 to 15 days. Adults lived at an average of 2.67 ± 0.18 days and preferred to lay eggs in adult female CSI. Other behavioral traits like adult emergence, feeding, host size preference, oviposition, and reproduction were observed. Host preference test was also conducted on *As. rigidus* and *As. destructor*. Based on the results, *Ap. chrysomphali* had a higher parasitism rate (25.13% ± 7.47) and laid more eggs (11.56 ± 3.38) in *As. destructor* than *As. rigidus*. In the latter host, higher offspring survivorship (61.03% ± 13.15) and more male-biased offspring (45.83% ± 16.40) were observed. However, there was no significant difference between the two host species in all parameters measured (percentage of parasitism, number of offspring, survivorship, and sex ratio). Spatial distribution of *Ap. chrysomphali* was also reported and shows that this parasitoid is present in most of the areas surveyed (63%) in the Philippines. These data support that *Ap. chrysomphali* can be a potential biological control agent against *As. destructor* infesting coconut.

Key words: *Aphytis chrysomphali*, *Aspidiotus destructor*, *Aspidiotus rigidus*, biology, coconut scale insect, parasitism

INTRODUCTION

The coconut industry in the Philippines was threatened by an introduced species of coconut scale insect (CSI), *Aspidiotus rigidus* Reyne (Hemiptera: Diaspididae), last 2010 to 2014. In the field, this species is observed together with *As. destructor* Signoret, a species already recorded in the country. This information is crucial in terms of its control, which is why a preliminary study on the preference of *Aphytis chrysomphali* between *As. rigidus* and *As. destructor* was conducted. However, the bio-ecological interaction between the two host species in the field is still unknown and beyond the scope of this study. In terms of economic damage, more than 2.6 million nut-bearing trees in the Southern Tagalog Region were infested (PCA, 2015). These pests are commonly found on the underside of the leaflets. They feed by sucking sap while secreting toxins from their salivary glands (Lal, 2004). Continuous feeding caused the drying up of leaflets resulting in initial yellowing and then turning brown in advanced cases. Different control tactics were integrated to manage these pests, which included pruning, fertilizer application, use of systemic insecticides, and release of biological control agents.

For long-term control, the use of biological control agents is of great promise. Among the hymenopterous parasitoids collected from CSI, *Ap. chrysomphali* is considered one of the potential biological control agents. This was reported and observed to attack CSI as early as 1900 (Huffaker & Messenger, 1976). In the Philippines, *Ap. chrysomphali* was reported to be collected from CSI (*As. destructor*), California red scale (CRS) (*Aonidiella aurantii*), and Florida red scale (*Chrysomphalus ficus*) infesting citrus in 1989 (Morallo-Rejesus et al., 1992 in Bilog-Obra et al., 2002). In Australia, *Ap. chrysomphali* is one of the primary parasitoids of CRS, *Ao. aurantii* that is one of the major insect pests of *Citrus* spp. (introduced and hybrid species) (Dao et. al., 2015).

Out of the eight species that were introduced and mass-released from 1943 to 1979, *Ap. chrysomphali*, *Comperiella bifasciata*, *Ap. melinus,* and *Prospaltella perniciosi* were the only species that permanently established against the CRS, *Ao. aurantii* in the lower Murray Valley of Australia (Furness et al., 1983). In Spain, a parasitism survey was conducted in citrus orchards in the center of the Valencia region, where they obtained *Ap. chrysomphali* in higher numbers compared to the introduced *Ap. melinus* (Rosen & De Bach, 1979). Some studies reported on the establishment and displacement of *Ap. chrysomphali.* A study concluded that *Ap. chrysomphali* was not displaced in many areas of eastern Spain as a parasitoid of CRS (Sorribas et al., 2008). In contrast, some reported about the displacement of *Ap. chrysomphali* by *Ap. melinus*, which was observed in Spain (Boyero et al., 2014; Sorribas et al., 2010), Cyprus (Orphanides, 1984), and Southern California (DeBach & Sundby, 1963). In 2016, at Tien Giang, Vietnam, the 25% parasitism level of *Ap. chrysomphali* was determined on *As. rigidus* in coconut as a host plant (Thi Dao et al., 2020). In this study, the potential ability of *Ap. chrysomphali* as a good biological control agent against *As. destructor* in coconut was assessed. Specifically, this study aimed to describe the biological and behavioral traits of *Ap. chrysomphali* using *As. destructor* as host, determine the preferred host of *Ap. chrysomphali* between *As. rigidus* and *As. destructor*, and determine the spatial distribution of *Ap. chrysomphali* in the Philippines.

MATERIALS AND METHODS

Rearing of CSI in host plant

Initial populations of *As. rigidus* and *As. destructor* were collected from Los Baños, Laguna. These two species were identified morphologically and verified molecularly (Caoili et al., 2014 in Beltran, 2016). The genomic DNA of *Ap. chrysomphali* was extracted using the Jena Bioscience Animal and Fungi DNA Preparation Kit. The partial cytochrome *c* oxidase subunit I (COI) was amplified for all specimens using forward primer LepF1 and reverse primer LepR1. Using the BLAST tool (blast.ncbi.nlm.nih.gov), the samples got 100% similarity with *Ap. chrysomphali*. The extraction and analysis were done and outsourced in the Genetics and Molecular Biology Division, Institute of Biological Sciences Building, University of the Philippines Los Baños. Each species of CSI was separately reared to prevent contamination, specifically for host preference study. CSI populations for biological studies were reared in 18-month-old coconut (synvar variety) seedlings with split leaves. The 2^{nd} instar and adult female CSI were used in observing the life cycle and behavior of *Ap. chrysomphali*. The protocol used by Tabibullah and Gabriel (1975) was used in mass rearing of CSI with some modifications. These include fastening/clipping of pieces of leaves that bear female CSI with eggs at the hatching stage and/or newly hatched eggs to the underside of the uninfested coconut leaves.

Rearing of *Ap. chrysomphali*

Population of *Ap. chrysomphali* was collected from coconut leaflets with parasitized CSI in a field from Los Baños, Laguna. These leaflets were transferred to emergence cages. Adults that emerged were collected and introduced to CSI-infested squash for mass rearing. Rearing was done under laboratory conditions (25—27°C and 30-50% RH). Representative samples from different areas were sent to a geneticist for molecular identification.

Biological and behavioral studies

The biological and behavioral traits of *Ap. chrysomphali* were studied using *As. destructor* as host instead of *As. rigidus*, which was reported to be the species causing the outbreak, because of the availability of the former species during that time. There was limited study and resources on how to collect, purify, and mass produce *As. rigidus* during that time.

Newly emerged adults of *Ap. chrysomphali* were collected and introduced to *As. destructor*-infested leaflets enclosed in test tubes plugged with cotton wool. The duration and description of the life cycle of *Ap. chrysomphali* and other biological attributes were studied using *As. destructor* as host and based on destructive sampling. A total of 121 individuals of parasitoids (i.e., egg-30; larva-36; pre-pupa-10; pupa-27; and adult-18) were monitored and sampled.

Host preference

Preliminary study on the host preference of *Ap. chrysomphali* was done through the dual-choice test using *As. rigidus* and *As. destructor* as hosts. This was done in the latter part of the entire study. Pupa from *Ap. chrysomphali*–infested seedlings was individually collected and initially placed in a 0.5mL-microcentrifuge tube. Adults that emerged were paired where each pair was introduced to two leaflets (*As. rigidus*–infested leaflet and *As. destructor*–infested leaflet) with the same number (average of 37.00 ± 5.36, ranges from 23 to 68 individuals per host per leaflet) and stage of CSI (adult female) enclosed in a test tube. The number of offspring, percentage of parasitism, survivorship, and sex ratio of *Ap. chrysomphali* were recorded.

Spatial distribution

Survey and collection of parasitized CSI on coconut and non-coconut hosts were done in 32 municipalities in 12 provinces of the country, namely Bataan, Cavite, Laguna, Batangas, Quezon, Palawan, Camarines Sur, Biliran, Davao Del Sur, Davao Del Norte, Sarangani, and Basilan. Samples were placed into flexi glass cages covered with black cloth and observed daily for adult emergence under laboratory conditions (25-27°C and 30-50%RH). The adults that emerged were collected from the mounted test tube of the cage and carefully examined for external anatomical characters. Test tube was mounted or placed on top of each flexi glass to allow the careful collection of emerged adults. The distribution of *Ap. chrysomphali* throughout the Philippine archipelago was recorded.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using the R program (R Core Team, 2021). Means were compared using t-test at 5% level of significance.

RESULTS AND DISCUSSION

Biology of *Ap. chrysomphali*

The developmental period and characteristics per stage of *Ap. chrysomphali* in *As. destructor* are shown in Figure 1. The egg is elliptical and translucent, laid singly, and deposited at the ventral side of the *As. destructor* body. Egg sizes ranged from 3–4 µm (Fig.1a). The larva is transparent white to opaque yellow. This can be recognized from the scale insect by their yellow guts.

Larva ranges from 4–6 µm (Fig. 1b). According to Rosen & DeBach (1979), three larval instars can be recognized in terms of the mandible size and shape and the number of spiracles. At this stage, the sex cannot be determined. The pre-pupa is similar in appearance to the larva except that it lacks gut coloration. Pre-pupa ranges from 5–6 µm (Fig. 1c). Pupa of *Ap. chrysomphali* has an exarate type of pupa. This changes in color from white to yellow as it matures. Eye color progresses from orange to red to black with age. Pupa ranges from 6–9 µm (Fig. 1d). At this stage, sex can now be determined. In the female, two plates are present ventrally near the tip of the abdomen whereas one plate is present in the male, and the tip of the abdomen is prominently notched. The adult head is hypognathous. It has a yellow body with three red ocelli arranged in a triangular position. Antennae are usually 6-segmented. Adult ranges from 7–12 µm (Fig. 1e) and lives at an average of 2.67 ± 0.18 days. This parasitoid species completed its development from egg to adult in 14 to 15 days. The developmental period per stage of *Ap. chrysomphali* is summarized in Fig. 1. Based on Beltran (2016), the developmental period (egg—adult) of female *As. destructor* is 43.4 days, while *As. rigidus* is 59.10 days. On the other hand, adult female longevity of *As. destructor* and *As. rigidus* is 32.68 ± 4.08 and 61.963 ± 9.55, respectively. Using this adult longevity data, *Ap. chrysomphali* can have approximately 2 and 4 generations using females *As. destructor* and *As. rigidus*, respectively. This also implies that *Ap. chrysomphali* has a shorter life cycle compared to its hosts. Moreover, other species like *Ap. chilensis, Ap. hispanicus,* and *Ap. coheni* have a developmental period of 18.6 days (Rosen & Eliraz, 1978), 16 days (Gerson, 1968), and 12 days (Avidov et al., 1970), respectively.

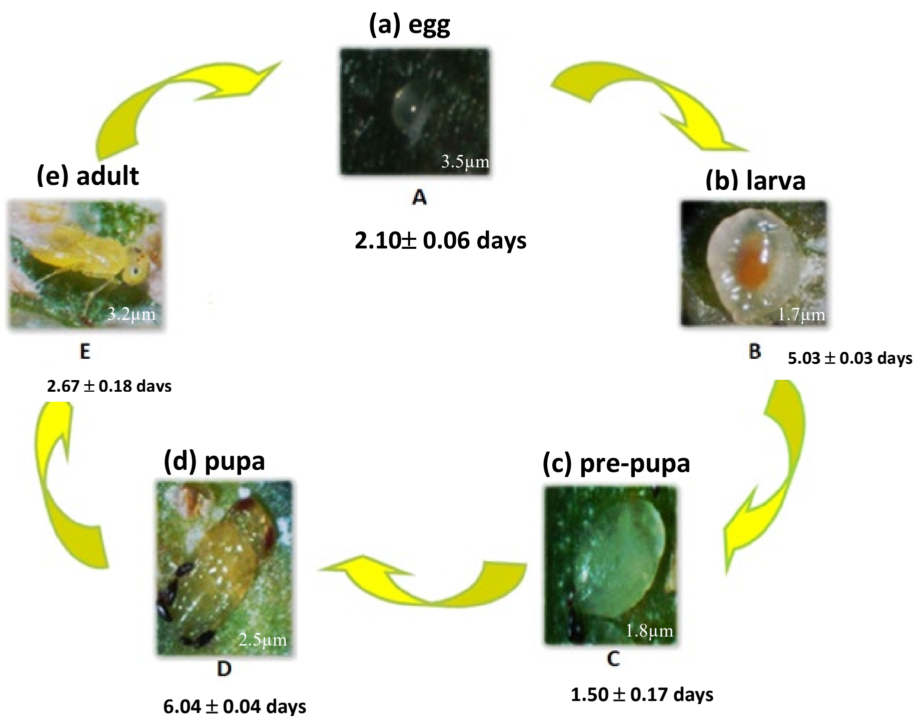

Figure 1. Life cycle and developmental period of *Ap. chrysomphali* on *As. destuctor* under laboratory conditions ((25-27 °C and 30-50% RH).

Behavior

Adult emergence and sign of parasitism. The adult was observed to be positively phototrophic and emerged from 6 a.m. to 10 a.m. by pushing the anterior part of the scale cover. Signs of *Ap. chrysomphali* parasitism included the parasitoid itself, meconial pellet (fecal matter, which is usually found in the posterior part of the scale cover), dried (parasitized) CSI host (Fig. 2), and sometimes created an exit hole. Related to this, a study conducted by Pekas et al. (2015) concluded that *Ap. chrysomphali* may be employing the CRS sex pheromone as a kairomone (allelochemical that evokes in the receiver a response that is adaptively favorable only to the receiver) for host location. This is one of the attributes of an efficient parasitoid which has good searching behavior.

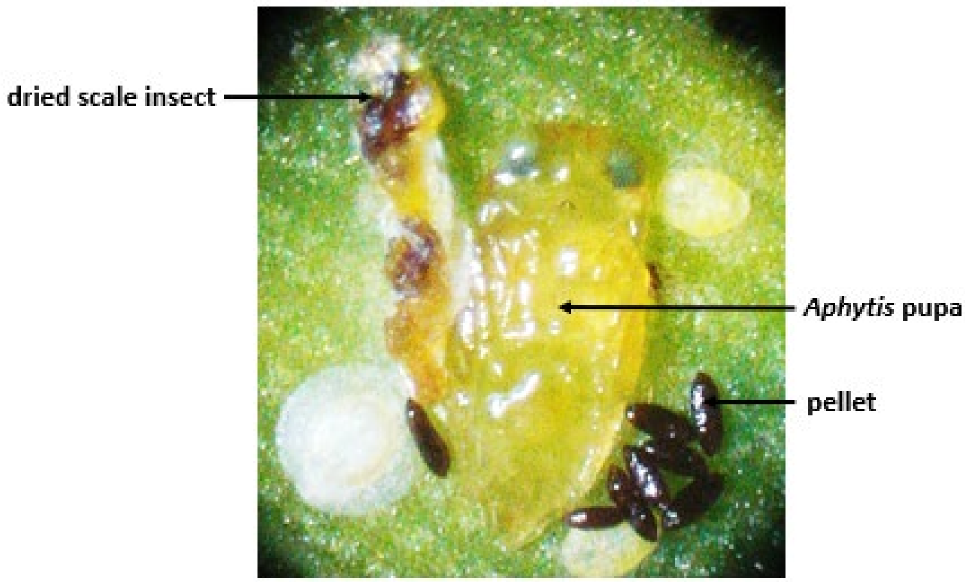

Figure 2. Signs of *Ap. chrysomphali* parasitism on *As. destructor.*

Feeding. Larvae fed on the body fluids of the scale insect. They started to excrete the gut material in the form of meconial pellets prior to the pre-pupal stage. As the larva continued to feed, the CSI body dried up and shrank until only the integument remained. Adult *Ap. chrysomphali* fed on smaller scale insects. It first probed the scale body and then fed on the body fluids that came out from the wounds. Host feeding is being done by the adult female to secure nutrients needed for egg maturation (synovigeny) for future reproduction (Heimpel & Rosenheim, 1995). Production of eggs is dependent on nutrition. This feeding also causes the mortality of CSI in the field.

Host size preference. *Ap. chrysomphali* parasitized 2nd instar and adult female of CSI, although it showed preference for the latter one. This is due to the larger size of adult female (1.08 mm body length and 0.94 mm body width) compared to the 2nd instar female (0.62mm body length and 0.53mm body width) (Beltran, 2016). This is one of the good attributes of an efficient parasitoid since it prefers a female host, which is responsible for reproduction. Based on the study conducted by Pekas et al. (2010), *Ap. chrysomphali* parasitized more heavily third instar females than 2nd instar females and males of CRS, *Ao. aurantia.* The size of developing offspring is affected by the number of offspring laid per host and host size/stage/age. Larger offspring developed when one egg was laid within a host, while smaller offspring developed when two or more eggs were laid within a host. On the other hand, the larger/older the host (e.g. adult female CSI), the larger the developing offspring, especially in 1 parasitoid egg: 1 host ratio. Larger hosts are considered of higher quality which are favorable for parasitoid development. The same trend was also observed in the study of Pekas (2011) on *Ap. chrysomphali* against CRS. Larger offspring is observed in a larger host. In the study of Pekas et al. (2010), percent parasitism, gregariousness, and offspring size of *Ap. chrysomphali* were positively related to host size. This parasitoid was not observed to parasitize male CSI because of its smaller size compared to female. This size of the host brings low quality of nutrition to the offspring of the parasitoid.

Oviposition and reproduction. Adult *Ap. chrysomphali* is observed to be a solitary or gregarious parasitoid. It first paralyzed the insect (e.g., *As. destructor*) before inserting its egg. Though adult female of *As. destructor* is considered immobile, it can still move in a way that it turns around during oviposition through its pygidium (Beltran, 2016). Through paralysis, it was not able to move its body. *Ap. chrysomphali* laid one egg or more per one host. Probably due to a lack of available hosts, different *Ap. chrysomphali* laid eggs on just one individual host resulting in superparasitism. This was also observed in the study of Pekas et al. (2010), wherein *Ap. chrysomphali* behaved as gregarious parasitoid depending on the host size. Also, this ectoparasitoid is considered as idiobiont since its host development is being arrested upon parasitism. This parasitoid exhibited both sexual and asexual reproduction (parthenogenesis).

Host preference

Ap. chrysomphali had a higher parasitism rate (25.13% ± 7.47) and laid more eggs (11.56 ± 3.38) in *As. destructor* than *As. rigidus* (Table 1). In the latter host, higher survivorship (61.03% ± 13.15) and more male-biased offsprings (45.83 ± 16.40) were observed. However, in all parameters used to determine the preference of *Ap. chrysomphali,* t-test showed no significant difference between *As. rigidus* and *As. destructor.* The frequency of oviposition to each host was also analyzed, and it showed that it laid eggs on the two hosts when present both.

However, the result from this preliminary study on the preference of *Ap. chrysomphali* is not conclusive. Additional trials and parameters must be done and measured to provide enough evidence to determine the preference of *Ap. chrysomphali*.

Table 1. Host preference of *Aphytis chrysomphali* Mercet between *Aspidiotus rigidus* Reyne and *As. destructor* Signoret using 23-68 individuals per host per leaflet.

Parameter	Host		t-test value*
	Aspidiotus rigidus	***Aspidiotus destructor***	
Number of offspring	5.89 ± 1.48	11.56 ± 3.38	0.1524
Parasitism (%)	12.68 ± 2.76	25.13 ± 7.47	0.1482
Survivorship (%)	61.03 ± 13.15	38.96 ± 12.38	0.2394
Sex ratio	5M:1F	1.25M:1F	-
Male (%)	(45.83 ± 16.40)	(37.04 ± 15.80)	0.7044
Female (%)	(9.72 ± 8.28)	(29.63 ± 14.79)	0.2572

*Means having t-test value less than 0.05 were significantly different with each other

Spatial distribution

Based on the samples collected from CSI-infested coconut hosts, *Ap. chrysomphali* was found throughout the country. Out of 32 municipalities surveyed, *Ap. chrysomphali* was observed in 20 municipalities (63%) (Figure 3). Nonetheless, whether *Ap. chrysomphali* is not present in other municipalities is not conclusive. Using the data presented, it shows that this species is present in almost all areas surveyed in the Philippines.

CSI (*As. rigidus* and *As. destructor*) were also observed to infest non-coconut plants/hosts. Based on the collected samples, *Ap. chrysomphali* was observed to parasitize CSI-infesting avocado (*Persea americana*), guava (*Psidium guajava*), pigeon pea (*Cajanus cajan*), and cashew (*Anacardium occidentale*).

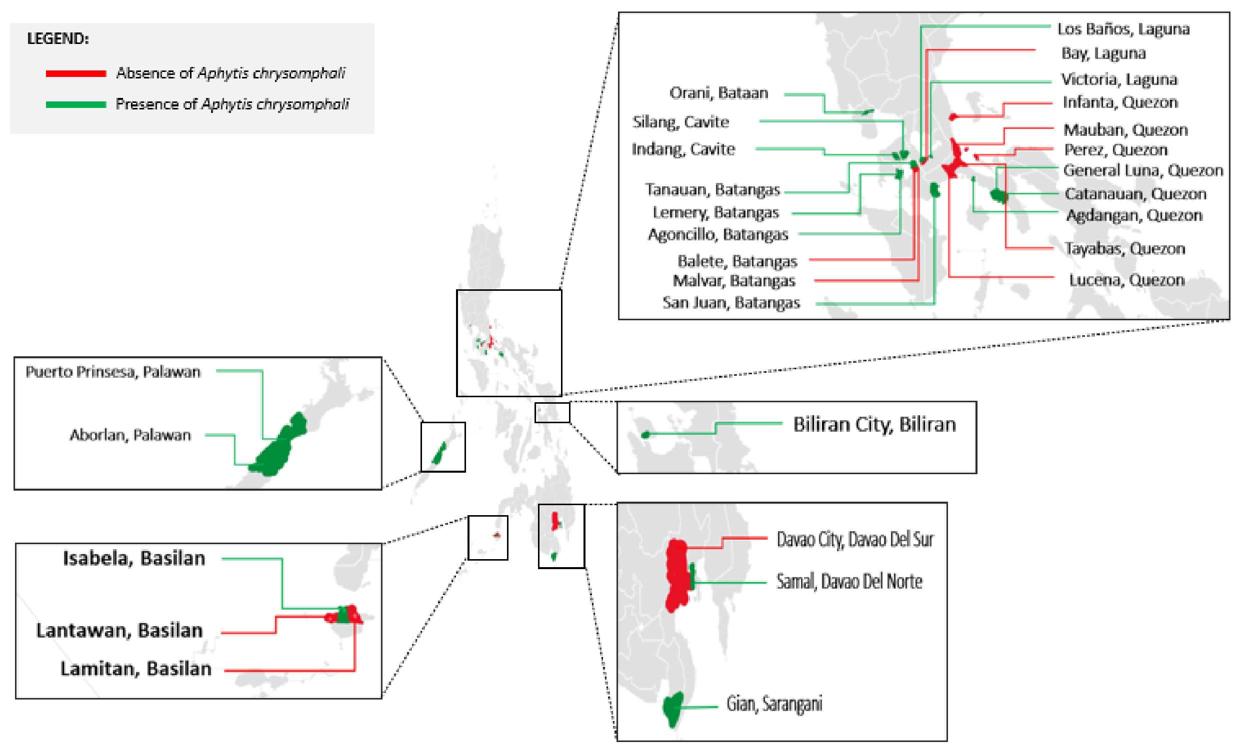

Figure 3. Distribution of *Ap. chrysomphali* in the Philippines based on collected CSI-infested coconut samples.

SUMMARY AND CONCLUSION

Ap. chrysomphali completed its development from egg to adult in 14 to 15 days. In synchrony with female CSI longevity, the population of *Ap. chrysomphali* can have approximately 2 and 4 generations on *As. destructor* and *As. rigidus*, respectively. Moreover, some behavioral traits (i.e., adult emergence, larval and adult feeding, and oviposition and reproduction) of *Ap. chrysomphali* were also observed. Adult emergence was observed around 6 a.m. to 10 a.m. They emerged by pushing scale cover or sometimes creating exit holes. In terms of feeding, both larvae and adults fed on CSI. The former fed on the body fluids until the host dried up, while the latter fed for egg development for future reproduction and oviposition. In terms of oviposition, it preferred to lay eggs in the adult stage of female CSI due to its larger size compared to 1st and 2nd instar. The size of developing offspring is affected by the age and stage of CSI and the number of offspring per scale. The larger the CSI, the larger the developing parasitoid offspring is. One or more *Ap. chrysomphali* offspring per CSI was also observed. Mating is common in *Ap. chrysomphali* adults, but sometimes parthenogenesis is observed. The following are the signs to monitor the parasitism of *Ap. chrysomphali* on CSI: (a) parasitoid itself inside the scale, (b) meconial pellet, (c) dried CSI, and/or (d) exit hole at times. A preliminary study on the preference of

Ap. chrysomphali between *As. rigidus* and *As. destructor* was also conducted. Four parameters were measured, namely percent parasitism, number of offspring, survivorship, and sex ratio. *Ap. chrysomphali* had a higher parasitism rate and fecundity in *As. destructor.* On the other hand, it showed higher survivorship and more male-biased offspring in *As. rigidus.* All these parameters provided no significant difference between *As. rigidus* and *As. destructor.* Thus, there is not enough evidence to determine the preference of *Ap. chrysomphali* between the two host species. More studies measuring other parameters (e.g. functional response) should be done. Moreover, the distribution of *Ap. chrysomphali* in some localities in the country was determined. Out of 33 municipalities surveyed, *Ap. chrysomphali* was observed to be present in 20 municipalities. These data support that *Ap. chrysomphali* can be a potential biological control agent against *As. destructor* infesting coconut.

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