BIODIVERSITAS
Volume 24, Number 3, March 2023
Pages: 1629-1634

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240335

Parasitoid of cassava mealybug, *Anagyrus lopezi* (Hymenoptera: Encyrtidae): Mummy size, adult emergence, sex ratio, and parasitization level

FATAYATUN NAIMAH[1], DEWI SARTIAMI[2,♥], NINA MARYANA[2], RULY ANWAR[2] , PUDJIANTO[2]
[1]Program of Entomology, Graduate School, Institut Pertanian Bogor. Jl. Kamper, Kampus Dramaga, Bogor 16680, West Java, Indonesia
[2]Department of Plant Protection, Faculty of Agriculture, Institut Pertanian Bogor. Jl. Kamper, Kampus Darmaga, Bogor 16680, West Java, Indonesia.
Tel.: +62-251-8629364, Fax.: +62-251-8629362, ♥email: dsartiami@apps.ipb.ac.id

Manuscript received: 12 December 2022. Revision accepted: 14 March 2023.

Abstract. *Naimah F, Sartiami D, Maryana N, Anwar R, Pudjianto. 2023. Parasitoid of cassava mealybug,* Anagyrus lopezi *(Hymenoptera: Encyrtidae): mummy size, adult emergence, sex ratio, and parasitization level. Biodiversitas 24: 1629-1634.* The presence of *Anagyrus lopezi* as a natural enemy can reduce the population of *Phenacoccus manihoti*. This parasitoid was introduced from Thailand to Indonesia in 2014 to control cassava mealybugs. This research aims to determine the mummy length and width, adult emergence time, sex ratio, and parasitization level. This study was conducted in July-November 2022, while parasitoids and mealybugs were obtained from Bogor, Indonesia. The size of mummies was measured under Dino-light digital microscope; the emergence time was counted for 24 hours within six days, and the sex ratio of parasitoids was calculated based on the number of males and females emergence. The parasitization level was observed from four female parasitoids at 120, 160, and 200 of host population densities. The study showed the length and width of female mummies (n: 100) were 0.71 mm and 1.64, while the length and width of male mummies (n: 100) were shorter than female with 1.00 mm and 2.18 mm. The peak time of adult emergence is *A. lopezi*, i.e., during the daytime at 09:01-12:00 AM and 03:01-06:00 PM. The sex ratio of parasitoids from the field and laboratory was 1:0.49, 1:1.05, and 1:1.09, respectively. The one-way ANOVA and Tukey's test at a 5% significance level showed that the host population density of 120 nymphs was significantly different from other densities, with a parasitization level of 66.7%.

Keywords: Cassava, mass-rearing, mealybugs, natural enemies, *Phenacoccus manihoti,* Pseudococcidae

INTRODUCTION

Cassava production was already obstructed by mealybugs infestation. The cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) is the most abundant and destructive species among other mealybugs which attack cassava plants in Indonesia (Wardani et al. 2019). Mealybug *P. manihoti* reached and invaded Indonesia Indonesia for the first time in 2010 (Muniappan et al. 2011) and shows a strong preference for cassava plants (CABI 2022). This pest's lack of effective control has caused losses with yield losses of up to 85% in Africa (Zeddies et al. 2001).

Biological control has often proven to be invasive species' most cost-effective management tool (Street 2015). One of the biological and environmentally friendly controls is using natural enemies, parasitoids. Successful control of cereal, vegetable crops, and other agricultural pests in China uses the role of parasitoids (Wang et al. 2019a). *Anagyrus lopezi* (Hymenoptera: Encyrtidae) is a parasitoid introduced to Indonesia for the first time and released to a cassava plantation in Bogor to control *P. manihoti* (Fanani et al. 2020). In the most recent development, 3000 *A. lopezi* wasps were released in a field in Bogor, western Java, Indonesia in September 2014, as a first step toward what might eventually become a nationwide mealybug control effort in Indonesia (Wyckhuys 2014). The recorded parasitoids spread to some regions of cassava plantations infested with *P. manihoti* showing a parasitization rate 25% in 2017 at Java Island. (Abdulchalek et al. 2017) and 59.1% in 2019 at East Java (Fanani et al. 2019).

Successful mass rearing of biological agents is a critical factor for the success of biological control programs. However, mass or laboratory rearing can affect insect performance through laboratory adaptation, inbreeding depression, deliberate selection, or direct rearing effects (Sorensen et al. 2012). Some biological factors as the sex ratio of *A. lopezi*; in the field, the sex ratio of *Epidinocarsis lopezi* became more male-biased at higher host densities (Dijken et al. 1991). The capability *of A. lopezi* cassava mealybugs has been recorded since the host entered the 2nd stage instar, and most prefer the 3rd instar (Adriani et al. 2020). The cassava mealybugs which have been successfully parasitized will be formed as pupae. This pupae look like a capsule and is called a mummy. The mummy helps protect the developing larval parasitoid (Daane et al. 2008). Adult parasitoids will appear through an opening of the tip of the mummy, while the size of the mummy will determine the sex of the parasitoid (Adriani et al. 2020). However, the measurement has not been done to differentiate the sex. The behavior of insect emergence from pupae occurs in a specific time, and emergence time is reflected due to host quality-driven development or host phenology (Rull et al. 2009); however, the adults of *A.*

lopezi time emerging are still unknown.

Mass rearing and production of parasitoid *A. lopezi* cannot be separated from the information on many aspects, i.e., the rearing technique, cage specification, and quality control under laboratory rearing conditions. This study was conducted to understand the factors that must be prepared and carried out before the mass release of parasitoids in the form of mealybug mummies. Furthermore, to support this program, information about the biological factors of parasitoids needs to be known. There is no data yet on the size of mummy male and female *A. lopezi*, the sex ratio in the field and laboratory, and the emergence time of adult *A. lopezi*. A study on the capability of parasitization of *A. lopezi* laboratory-scale has been reported by Maharani et al. (2019) by 35% and Fanani et al. (2020) by 17.04%. However, these results have not met the optimum rearing requirements. This research aims to determine the mummy length and width, adult emergence timing from mummies, sex ratio, and level of parasitization.

MATERIALS AND METHODS

The research was conducted from July to November 2022 at the Laboratory of Insect Biosystematics and Bionomi and Insect Ecology, Department of Plant Protection, Institut Pertanian Bogor (IPB). The average daily temperature and relative humidity were 26 ± 1.1ºC and 52 ± 9.2%, respectively, at the time of the study. Samples of mealybugs and parasitoids were obtained from the cassava fields of Sukaraja, Cimahpar, and Sentul Villages, North Bogor District, Bogor, West Java, Indonesia. The rearing of *P. manihoti* and parasitoid *A. lopezi* used cassava cuttings.

Host plant and mealybugs cassava rearing

The cassava variety used is the Manggu variety. About 15 cm of cassava cuttings were put into plastic cups (d: 15 cm; t: 9.5 cm) containing AB mix nutrients covered with styrofoam and a circular space in the middle to place the cuttings stand. Cassava cuttings are allowed to grow until leaf buds appear.

The rearing of mealybugs follows the rearing method of Adriani et al. (2020). One imago of *P. manihoti* is infested on cassava cuttings aged two weeks. Rearing is carried out inside a cage measuring (l: 75 cm; w: 51 cm; h: 54 cm) containing ten cassava cuttings. Rearing of *P. manihoti* is carried out up to *P. manihoti* reaches the 3rd instar, which will be used in this study.

Rearing of parasitoids

The rearing of parasitoids follows Fanani et al.'s method (2020). Adult *A. lopezi* males and females were put into a cage containing cassava plants infested with 3rd instar of *P. manihoti*. The cage was made (l: 75 cm; w: 51 cm; h: 54 cm) with walls made of organdy fabric and plastic inside, which contains a cotton ball containing a 10% honey solution. Two weeks after exposure, the mealybugs mummified are collected and put into the Eppendorf tube separately. Rearing was carried out under temperature and relative humidity conditions of 26 ± 1.1ºC and 52 ± 9.2%, respectively, and irradiation of 12 hours of light and 12 hours of darkness.

Mummy size of *Anagyrus lopezi*

Mummy *P. manihoti* from the rearing of parasitoids is collected and put into Eppendorf. Mealybug mummies are measured using the Dino-lite app based on the size of the mummy's length. After measurements, observations of the daily appearance of imago from each sample of mealybug mummies were carried out.

Emergence time of *Anagyrus lopezi*

Mealybug mummies formed from the rearing results are collected and put into Eppendorf separately and arranged on styrofoam. Observations were made over six days with an observation time interval of 3/24 hours under light conditions and relative temperature and humidity of 26 ± 1.1ºC and 52 ± 9.2%, respectively. Adults' parasitoid emergence is recorded every three hours and distinguished between males and females.

Sex ratio of *Anagyrus lopezi*

Samples of mealybug mummies from cassava fields in Sukaraja and laboratory rearing were collected and put into Eppendorf separately. Eppendorf is composed of styrofoam to facilitate the observation of the emergence of adult parasitoids. The emergence of adult parasitoids was recorded. The calculation of the sex ratio is carried out based on a comparison of the number of male and female adult emergence.

Parasitization level at different host densities

A total of four copulated adult female parasitoids are put into cages measuring 9x30 cm with a lid in the form of organdy cloth mounted on top of a plastic cup measuring 15x9.5 cm containing nutrients and cassava cuttings. A 10% honey solution is applied to the outside of the organdy cloth daily. This test used a non-factorial, completely randomized design (CRD) with different levels of 3rd instar of *P. manihoti* density consisting of 120, 160, and 200, each repeated five times. This test was carried out for two weeks until a mealybug mummy was formed under a temperature and relative humidity of 26 ± 1.1ºC and 52 ± 9.2%, respectively. The number of mummies of the formed mealybug is then recorded.

Data analysis

A one-way ANOVA analysis was performed to determine the effect of host density on the parasitization level of *A. lopezi* and continued with the Tukey test at a 5% significance level. In addition, the test results on measuring the mummy, sex ratio, and adult emergence time were tabulated in Ms. Excel 2016. All data processing is carried out using Ms. Excel 2016 and SAS. 9.4.

RESULTS AND DISCUSSION

Phenacoccus manihoti is often found at the growing point of cassava plants, young leaves, and young stems. Nymphs will suck sap young cassava plant parts or on the buds. Symptoms of attack are characterized by little and internode distortion. However, high populations can result in leaf drop, shoot death, and severe defoliation. Figure 1 shows the symptoms in laboratory mealybug rearing.

Mummy size of male and female parasitoids

Figure 2.A shows that the size of male mummy's length and width is smaller than that of the female mummy. The length of the female mummy's minimum value is 1.57 mm, and the maximum length value is 2.70 mm. The length of the male mummy is smaller than the length of the female mummy with a minimum length value of the male mummy of 1.33 mm and a maximum length of 2.44 mm. Figure 2B shows that the width of the female mummy is greater than the width of the male mummy, with a minimum width value of 0.64 and a maximum width value of 1.35 mm. The median values of both the width and length of the male and female mummies are different, with the median values of widths of 0.70 mm and 1.001 mm, respectively, and the median values of lengths of 1.64 mm and 2.18 mm, respectively. Figure 3 shows variation of mummies size.

Emergence time of *Anagyrus lopezi*

Based on the data on the emergence of adult *A. lopezi*, the number of adults with the highest occurrence rate is at 09:01-12:00 AM, followed by the appearance time at 03:01-06:00 PM and 06:01-09:00 PM. The lowest time of adult *A. lopezi* emergence is at 09:01-00:00 PM and 00:01-03:00 AM. The emergence of adult *A. lopezi* increases at 06:01-09:00 AM, and the highest increases at 09:01-12:00 AM and 03:01-06:00 PM and decreases with time (Figure 4).

Sex ratio of male and female parasitoids

The sex ratio of male and female parasitoids obtained from the Sentul and Sukaraja fields showed differences. The results of the acquisition of mummy samples from the field in Sentul showed that the sex ratio of males was higher than females, with a ratio of males to females of 2.03:1 from 348 mealybug mummies. The parasitoid sex ratio value from the acquisition from the field in Sukaraja shows that females are slightly higher than males with a male: female ratio of 1:1.05, respectively, from 306 mealybug mummies. The difference between the sex ratio of males in the two field locations, Sentul and Sukaraja, was 1.03. The sex ratio obtained from laboratory propagation showed that females were higher than males, with a male: female ratio of 1:1.09, respectively. All data showed in Table 1.

Parasitization level of *Anagyrus lopezi* on various host densities

Based on the analysis using the 5% Tukey test, we found that the *A. lopezi* infestation rate at a density of 120 hosts was significantly different from the density of 160 hosts and not significantly different from the density of 200 hosts (Table 2). Four copulated female adults, *A. lopezi* at a density of 120 hosts, could infest up to 66.6%, and the number of mealybug mummies formed from each parasitoid was up to 20 mummies. The density of 160 hosts shows the lowest parasitization level at 28.5%, while the average number of mummies produced by each parasitoid was 11.4. The parasitization level at a density of 200 hosts was not significantly different from 120 hosts, with a value of 31.4% and an average of 15.7 mummies formed by each parasitoid.

Figure 1. The symptoms caused by *Phenacoccus manihoti*

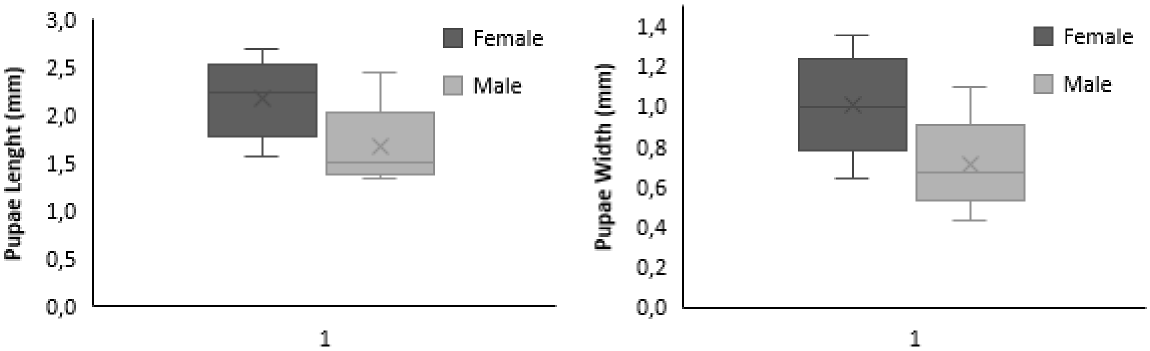

Figure 2. A. The size of the mummy length of the parasitoid *Anagyrus lopezi* male and female; B. The size of the width of the mummy of the parasitoid *Anagyrus lopezi* male and female

Figure 3. The mummified mealybugs

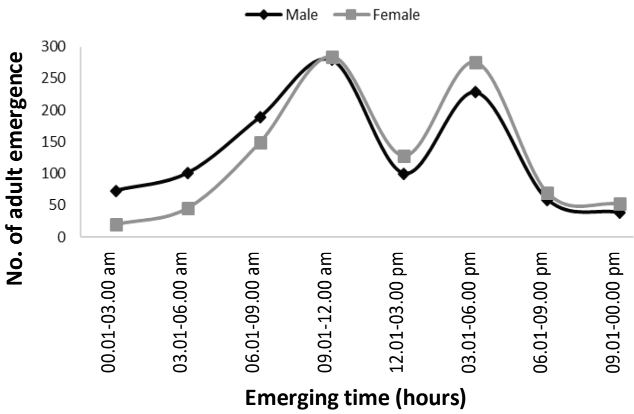

Figure 4. Emergence time of adult *Anagyrus lopezi*

Table 1. Parasitoid sex ratio *Anagyrus lopezi* in the field and laboratory

Sample Origin	N	Sex Ratio	
		Male	Female
Fields			
Sentul	348	2.03	1
Sukaraja	306	1	1.05
Laboratory	3682	1	1.09

Table 2. Parasitization level of *Anagyrus lopezi* at various host numbers

Number of female parasitoids	Host density	Parasitization (%)	Number of mealybug mummies per parasitoids
4	120	66.6^{a*}	20^{a}
4	160	28.5^{b}	11.4^{b}
4	200	31.4^{b}	15.7^{ab}

Note: *Mean in a column followed by different letters showed a significance at $p \leq 0.05$

Discussion

Phenacoccus manihoti was the most destructive pest, with high yield losses of up to 85%. It is caused by *P. manihoti* feeding on cassava plants, affecting terminal shoots distortion, yellowing and curling of leaves, reduced internodes, and weakening of stems used for crop rearing (Mani and Shirajavu 2016). Figure 1 shows that the symptoms caused curly leaves and the weakening of the cassava stem. The colony of *P. manihoti* was assembled on one spot together in every node or under leaves. Because of the high losses and damage, *P. manihoti* must be controlled to reduce the yield losses. Therefore, the biological control of *P. manihoti* was introduced in other countries. One of the biological controls of *P. manihoti* is using natural enemies, parasitoids. Therefore *A. lopezi* was known as an effective parasitoid controlling cassava mealybugs. FAO collaborated with national organizations to spread *A. lopezi* in Laos (2011), Cambodia (2012), and Vietnam (2013), and most release locations in mainland Southeast Asia have noticed significant development of *A. lopezi*, which greatly controls *P. manihoti* populations (Lim and Moekchantoek 2013). Therefore, mass rearing and production of parasitoids are carried out to provide biological stocks for controlling cassava mealybugs. Several aspects need to be known in mass rearing and production, which is the biological aspect of *A. lopezi*.

Female parasitoids' mummy is larger than male parasitoids (Figure 2). It is caused by the adult female parasitoid laying eggs on a large host (≤ instar 3). According to Sarkar et al. (2015), many parasitoid wasp species lay male and female eggs in various hosts: male eggs are frequently laid in smaller hosts, and female eggs in larger hosts. The mummy size of female parasitoids is larger, likely due to the quality factor and size of the host. Parent size is positively correlated with host qualities. Female parasitoids will lay fewer eggs in low-quality hosts and develop as male offspring (Nurkomar et al. 2021). It can be concluded that the host's size strongly affects the parasitoid's size, with large parasitoids emerging from large hosts (Ueno 2015). In larger hosts, parasitoids lay fertilized eggs (which produce females); in smaller hosts, parasitoids lay non-fertilized eggs (which produce males). In some conditions, females died at a higher rate in smaller hosts because they did not reach pupal weight (Mohamad et al. 2015). The host instar also affects the parasitoid's pupa size of the resulting progeny. The proportion of male parasitoids will decrease as the host's size or stage increases (Adriani et al. 2020). Female and male *A. vexans* emerging from parasitized hosts at the third instar were significantly larger than in other stages. This may explain the preference for the third instar and the female-biased sex ratio, as size is usually positively correlated with higher fitness, especially in females (Bertschy et al. 2000). Grouping based on the size of the mummy between male and female parasitoids is carried out to determine the average size of the mummy of the parasitoids. That will come out male or female so that the number of mummies released into the field in each release medium can be compared.

The highest emergence time of adult *A. lopezi* at 09:01-12:00 AM (Figure 4). Increasing occurrences of adult *A. lopezi* from 06:01-09:00 AM to 09:01-12:00 AM may be caused by the temperature factor in the morning to noon being relatively increased, and the relative humidity decreases. The result is similar to *Anagrus* sp. the peak emergence time is 10:00 AM (Meilin et al. 2012), and the parasitoids are active from 5:00 AM to 01:00 PM (Haryati et al. 2017). Parasitoids support this as diurnal insects that move daily to forage or continue their offspring (Fantinou et al. 1998). The timing of adult emergence is important for the mass release of biocontrol agents to determine the exact time of harvesting of mummies and release before the emergence of adults from parasitoids useful in biological control programs. The emergence of adults in the morning has been reported in many diurnal insects, and this behavior can result from adaptation to certain constraints (Karpova 2006). Generally, parasitoids are active in the daytime due to parasitism or migration, such as *Telenomus remus*, that mainly active during the daytime (Chen et al. 2021). Mild climatic conditions in the morning (high humidity and low temperature) facilitate the emergence of parasitoids and other activities such as wing expansion, mating, and dispersion, which is reflected in the rhythm of emergence in four species of *Trichogramma* (Wang et al. 2019b).

The sex ratio of *A. lopezi* from the Sentul and Sukaraja showed a difference (Table 1). The difference in the number of mealybug mummies obtained from Sentul is more than Sukaraja. Dijken et al. (1991) stated that the sex ratio of *Epidinocarsis lopezi* in the cassava field is biased in males, with males and females each 1:0.5 at high host density and females biased in low host density. So, the result following our results, the sex ratio of *A. lopezi* from Sentul, with the number of mealybugs mummies obtained, is 348 mummies (Table 1). It can be indicated that a large number of inbreeding adult parasitoids are produced, or when male reproductive return is low, females are thought to reduce the number of male offspring in a structured population (Gottlieb et al. 2014). The sex ratio of *A. lopezi* obtained from Sukaraja is biased in females, with the male and female value of 1:1.0.5. The sex ratio of male and female parasitoids at Bali showed the value of male and female, 1:1.33 (Supartha et al. 2020), so it can be said, the host densities of the parasitoid in the field are low. The host availability, such host stage available, can affect the parasitoid's sex ratio. The host stage affects parasitoid progeny, with the first instar of the mealybug producing a male parasitoid proportion value of 0.90. As the host stage increase, the female progenies are produced in a proportion value of 0.74 (Adriani et al. 2020). The offspring sex ratio (male ratio) decreases with increasing host size. The bigger hosts produce the bigger wasps. Mackauer and Chow (2016) state female parasitoids may change the sex allocation of their offspring depending on the number and quality of hosts available. Generally, the sex ratio theory assumes that the mother controls the offspring's sex allocation (Ueno 2014). The sex ratio tends to be female-biased is an important aspect of biological control, especially the inundation biological control factor, where females only carry out the control of the host population. The bias of the sex ratio in females can increase parasitization because only female adults can kill the target pest through parasitism and feeding. Therefore, the percentage of female adult parasitoids was the most crucial factor in pest biological control efficacy (Wang et al. 2021). Sex allocation determines the sex ratio, and the parasitoid will determine to lay eggs on the appropriate host regarding size, nutrition, and host stage. Host selection behavior is crucial in determining the sex ratio of arrhenotokous parasitoids. Host quality influences immature development and the subsequent fitness of the emerging parasitoid, with implications regarding the offspring sex ratio (Faharani et al. 2016).

The host density affects the number of parasitized hosts (Table 2). The uneven distribution of mealybug colonies on plants can cause this. Based on the theory, one imago *Anagyrus* sp. can parasitize ±20 hosts. Thus, our tests showed that at 120 host densities, four female *A. lopezi* parasitized until 66.6%, or reaching the number means 80 mummies. Those reached the optimum number per parasitoid at 20 mummies. The result was the same as that from Fanani et al. (2020), with 120 hosts, one adult female *A. lopezi* reaching the number parasitism of mealybugs up to 20.2. As the density of parasitoids increases in an area, the finding on females spends more time interacting with the specific one than looking for its host (Saini and Sharma 2018). The competition among foraging parasitoids can reduce the rate of parasitization (Lou et al. 2013). The distribution of host colonies is thought to affect the level of parasitization. That is because individuals in one host colony are stacked on top of each other and, on average, are found on the underside of cassava leaves, and on average, each plant shoot has 38.67 to 48.55 mealybugs (Rebu and Rauf 2018). On the search time to host, the parasitoid is challenging to reach the host stacked at the bottom of the colony. Thus, the low parasitism level at 160 host densities is due to the host movement and accumulation at one node. That might be owing to the parasitoid ovipositor not reaching its host at that depth (Liu et al. 2022). The number of parasitoids developed per host is one of the most critical factors influencing adult body size and future reproductive success (Samkova et al. 2019). Based on that, the number of produced parasitoids is the same as that of formed mealybug mummies. This solitary parasitoid needed an optimum number of hosts for biocontrol agent multiplication.

This study has provided information regarding several biological factors, such as the sex ratio of male *A. lopezi* and more samples from the field, while female *A. lopezi* is more than the result of laboratory rearing. The mummy size of males *A. lopezi* is relatively smaller than that of females, and the optimum number of host densities obtained from this study's results is 120 hosts. Based on the time of appearance of imago *A. lopezi*, it was observed that the optimal time for harvesting and releasing mealybug mummies into the field is the morning and evening before the peak of the appearance of imago *A. lopezi*.

ACKNOWLEDGEMENTS

The Ministry of Education, Culture, Research and Technology, The Republic of Indonesia supported this research under the PDKN (*Penelitian Dasar Kompetitif Nasional*) program, Grant no.: 082/E5/PG.02.00.PT/2022 and 3742/IT3.L1/ PT.01.03/P/B/2022.

REFERENCES

Abdulchalek B, Rauf A, Pudjianto. 2017. Kutu putih singkong, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae): Persebaran geografi di Pulau Jawa dan rintisan pengendalian hayati. Jurnal Hama Penyakit Tumbuhan Tropika 17 (1): 1-8. DOI: 10.23960/j. Hptt.1171-8. [Indonesian]

Adriani E, Rauf A, Pudjianto. 2020. Influence of host stage on oviposition, development, and sex ratio of *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae), a parasitoid of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae). Jurnal Hama Penyakit Tumbuhan Tropika 20 (2): 130-139. DOI: 10.23960/j.hptt.220130-139. [Indonesian]

Bertschy C, Turlings TCJ, Bellotti A, Dorn S. 2000. Host stage preference and sex allocation in *Aenasius vexans*, an encyrtid parasitoid of cassava mealybug. Entomol Exp Appl 95 (3): 283-291. DOI: 10.1046/j.1570-7458.2000.00667.x.

CABI. 2022. Invasive Species Compendium. CABI International, Wallingford, UK.

Chen W, Weng Q, Nie R, Zhang H, Jing X, Wang M, Li Y, Mao J, Zhang L. 2021. Optimizing photoperiod, exposure time, and host-to-parasitoid ratio for mass-rearing of *Telenomus remus*, an egg parasitoid of *Spodoptera frugiperda*, on *Spodoptera litura* eggs. Insects 12: 1050-1064. DOI: 10.3390/insects12121050.

Daane K, Cooper M, Triapitsyn S, Walton V, Yokota G, Haviland D, Bentley W, Godfrey K, Wunderlich L. 2008. Vineyard managers and researchers seek sustainable solutions for mealybugs, a changing pest complex. Calif Agr 62 (4): 167-176. DOI: 10.3733/ca.v062n04p167.

Dijken M, Neuenschwander P, Alphen J, Hammond W. 1991. Sex ratios in field populations of *Epidinocarsis lopezi*, an exotic parasitoid of the cassava mealybug, in Africa. Ecol Entomol 16 (2): 233-240. DOI: 10.1111/j.1365-2311.1991.tb00213.x.

Faharani KH, Ashouri A, Zibaee A, Abroon P, Alford L. 2016. The effect of host nutritional quality on multiple components of Trichogramma brassicae fitness. Bull Entomol Res 106 (5) : 633–641. DOI: 10.1017/S000748531600033X.

Fanani MZ, Rauf A, Maryana N, Nurmansyah A, Hindayana D. 2019. Geographic distribution of the invasive mealybug *Phenacoccus manihoti* and its introduced parasitoid *Anagyrus lopezi* in parts of Indonesia. Biodiversitas 20 (12): 3751-3757. DOI: 10.13057/biodiv/d210601.

Fanani MZ, Rauf A, Maryana N, Nurmansyah A, Hindayana D. 2020. Parasitism disruption by ants of *Anagyrus lopezi* (Hymenoptera: Encyrtidae), parasitoid of cassava mealybug. Biodiversitas 21 (6): 2337-2343. DOI: 10.1305/biodiv/d210601.

Fantinou AA, Alexandri MP, Tsitsipis JA. 1998. Adult emergence rhythm of the egg-parasitoid *Telenomus busseolae*. Biol Control 43: 141-151. DOI: 10.1023/A:1009984318289.

Gottlieb D, Lubin Y, Harari AR. 2014. The effect of female mating status on male offspring traits. Behav Ecol Sociobiol 68: 701-710. DOI: 10.1007/s00265-014-1683-1.

Haryati S, Trisyono Y, Witjaksono. 2017. Parasitism of the rice brown planthopper eggs in various periods of time of the day. Jurnal Perlindungan Tanaman Indonesia 20 (1): 28-35. DOI: 10.22146/jpti.16621. [Indonesian]

Karpova SG. 2006. The role of endogenous and exogenous factors in regulation of synchronous emergence of *Trichogramma embryophagum* Hartig and *T. principium* Sug. et Sor. (Hymenoptera : Trichogrammatidae). Entomol Rev 86 (3): 252-263. DOI: 10.1134/S001387380603002X.

Lim GS, Moekchantuk T. 2013. FAO TCP Project: Capacity Building for Spread Prevention and Management of Cassava Pink Mealybug in the Greater Mekong Subregion, Ho Chi Minh City, Vietnam. www.fao.org/fileadmin/templates/rap/files/meetings/2013/141104-report-casava.pdf.

Liu JF, Cheng XW, Atif I, Hai YZ, Mao FY. 2022. Effects of host ages and release strategies on the performance of the pupal parasitoid *Spalangia endius* on the melon fly *Bactrocera cucurbitae*. Agric 12 (10): 1629. DOI: 10.3390/agriculture12101629.

Lou YG, Zhang GR, Zhang WQ, Hu Y, Zhang J. 2013. Biological control of rice insect pests in China. Biol Control 68: 103-116. DOI: 10.1016/j.biocontrol.2013.06.011.

Mackauer M, Chow A. 2016. Females of the parasitoid wasp, *Dendrocerus carpenteri* (Hymenoptera: Megaspilidae), adjust offspring sex allocation when competing for hosts. Eur J Entomol 113: 542-550. DOI: 10.14411/eje.2016.074.

Maharani JS, Rauf A, Maryana N. 2019. Masa hidup imago, progeni dan kemampuan parasitasi *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae), parasitoid kutu putih singkong. Jurnal Entomologi Indonesia 16 (3): 138-150. DOI: 10.5994/jei.16.3.138. [Indonesian]

Mani M, Shirajavu C. 2016. Mealybugs and Their Management in Agricultural and Horticultural Crops. Springer, New Delhi.

Meilin A, Trisyono YA, Martono E, Buchori D. 2012. Mass-rearing technique of *Anagrus nilaparvatae* (Pang et Wang) (Hymenoptera: Mymaridae) using plastic box. Jurnal Entomologi Indonesia 9 (1): 7-13. DOI: 10.5994/jei.9.1.7. [Indonesian]

Mohamad F, Mansour M, Ramadan A. 2015. Effects of biological and environmental factors on sex ratio in *Ascogaster quadridentata* Wesmael (Hymenoptera: Braconidae), a parasitoid of *Cydia pomonella* L. (Torticidae). J Plant Prot Res 55 (2): 151-155. DOI: 10.1515/jppr-2015-0019.

Muniappan R, Shepard BM, Watson GW, Carner GR, Rauf A, Sartiami D, Hidayat P, Afun JVK, Goergen G, Ziaur RAKM. 2011. New records of invasive insects (Hemiptera: Sternorrhyncha) in Southeast Asia and West Africa. J Agric Urban Entomol 26 (4): 167-174. DOI: 10.3954/1523-5475-26.4.167.

Nurkomar I, Azhar A, Buchori D. 2021. Sex allocation and field population sex ratio of *Apanteles taragamae* Viereck (Hymenoptera: Braconidae), a larval parasitoid of the cucumber moth *Diaphania indica* Saunders (Lepidoptera: Crambidae). De Gruyter 6: 673-681. DOI: 10.1515/opag-2021-0045.

Rebu JU, Rauf A. 2018. Survei hama eksotik *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) pada tanaman singkong di Kabupaten Kupang. Prosiding Seminar Nasional Pertanian ke V Pengelolaan Pertanian Lahan Kering Berkelanjutan untuk Menunjang Kedaulatan Pangan. Kupang, 26 Oktober 2018. [Indonesian]

Rull J, Wharton R, Feder JL, Guillén L, Sivinski J, Forbes A, Aluja M. 2009. Latitudinal variation in parasitoid guild composition and parasitism rates of North American hawthorn infesting rhagoletis. Environ Entomol 38 (3): 588-99. DOI: 10.1603/022.038.0310.

Saini A, Sharma PL. 2018. Functional response and mutual interference of *Cotesia vestalis* (Hymenoptera: Braconidae) on *Plutella xylostella* (Lepidoptera: Plutellidae). J Entomol Sci 53 (2): 162-170. DOI: 10.18474/JES17-36.1.

Samkova A, Hadrava J, Skuhrovec J, Jansta P. 2019. Host population density and presence of predators as key factors influencing the number of gregarious parasitoid *Anaphes favipes* off spring. Sci Rep 9 (1): 6081. DOI: 10.1038/s41598-019-42503-4.

Sarkar M, Suasaard W, Uraichuen S. 2015. Host stage preference and suitability of *Allotropa suasaardi* Sarkar & Polaszek (Hymenoptera: Platygasteridae), a newly identified parasitoid of pink cassava mealybug, *Phenacoccus manihoti* (Homoptera: Pseudococcidae). Songklanakarin J Sci Technol 37 (4): 381-387.

Sorensen JG, Addison MF, Terblanche JS. 2012. Mass-rearing of insects for pest management: Challenges, synergies and advances from evolutionary physiology. Crop Pro 38: 87-94. DOI: 10.1016/j.cropro.2012.03.023.

Street C. 2015. Enhancing the Effectiveness of Biological Control Programs of Invasive Species by Utilizing an Integrated Pest Management Approach. Invasive Species Advisory Committee. https://www.doi.gov/sites/doi.gov/files/uploads/isac_biocontrols_white_paper_rev.pdf.

Supartha IW, Yudha IKW, Wiradana PA, Susila IW. 2020. Response of parasitoids to invasive pest *Phenacoccus manihoti* Matile Ferrero (Hemiptera: Pseudococcidae) on cassava crop in Bali, Indonesia. Biodiversitas 21 (10): 4543-4549. DOI: 10.13057/biodiv/d211011.

Ueno T. 2014. Age-dependent constraints of sex allocation in a parasitoid wasp. Psyche 2014: 363174. DOI: 10.1155/2014/363174.

Ueno T. 2015. Effects of host size and laboratory rearing on offspring development and sex ratio in the solitary parasitoid *Agrothereutes lanceolatus* (Hymenoptera: Ichneumonidae). Eur J Entomol 112 (2): 281-287. DOI: 10.14411/eje.2015.048.

Wang Z, Liu YQ, Shi M, Huang JH, Chen X. 2019a. Parasitoid wasps as effective biological control agents. J Integ Agric 18 (4): 705-715. DOI: 10.1016/S2095-3119(18)62078-7.

Wang Y, Xiang M, Hou YY, Yang X, Dai H, Li J, Zang LS. 2019b. Impact of egg deposition period on the timing of adult emergence in *Trichogramma* parasitoids. Entomol General 39 (3-4): 339-346. DOI: 10.1127/entomologia/2019/0896.

Wang S, Libo W, Jiawen L, Dayu Z, Tongxian L. 2021. Multiple mating of *Aphelinus asychis* enhance the number of female progeny but shorten the longevity. Insects 12 (9): 823. DOI: 10.3390/insects12090823.

Wardani N, Rauf A, Winasa IW, Santoso S. 2019. Effect of invasive pest *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in cassava. Intl J Environ Agric Biotechnol 4: 1440-1445. DOI: 10.22161/ijeab.45.24.

Wyckhuys KA. 2014. Parasitoids introduced into Indonesia: Part of a region-wide campaign to tackle emerging cassava pests and diseases. Biocontrol News Inform 35 (4): 29-38.

Zeddies J, Schaab RP, Neuenschwander P, Herren HR. 2001. Economics of biological control of cassava mealybug in Africa. Agric Ecol 24 (2): 209-219. DOI: 10.1111/j.1574-0862.2001.tb00024.x.