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Suppression of the Cassava Mealybug Populations, *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) By Natural Enemies

 M uhammad Zainal Fanani $^{1^*}$, Aunu Rauf 2 , Nina Maryana 2 , Ali Nurmansyah 2 , Dadan Hindayana 2 , Arifah Rahayu 1 , *Setyono Setyono¹ , Martin Roestamy¹*

¹ Universitas Djuanda, Jl. Tol Ciawi 1, Kotak Pos 35 Bogor 16720, Indonesia 2 Institut Pertanian Bogor, Jl. Kamper, Kampus IPB Darmaga, Wing 7 Level 5, Bogor 16680, Indonesia *Correspondence: E-mail: muhammad.zainal@unida.ac.id

The most dominant mealybug species found on cassava is *Phenacoccus manihoti*. Parasitoid *Anagyrus lopezi* is a biological agent of *P. manihoti* in many countries. Monitoring the incidence and population of mealybug and the percentage of parasitization were done, as well as the population of ants and predator insects for 12 consecutive months. The highest incidence and attack rate of mealybugs were 26 and 64%, respectively, during the dry season. *A. lopezi* was able to suppress the population of *P. manihoti* between 2-17 individuals per plant with a parasitization rate varied from 12 to 46%. Our study showed that the parasitization rate of *A. lopezi* increased significantly with the increase in mealybug density. Predators *P. ramburi* and *C. montrouzieri* were found in low populations and have not had a significant impact on the mealybug population. Our study demonstrated the important role of natural enemies in suppressing the mealybug population on cassava.

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1. INTRODUCTION

Cassava mealybugs, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) which are the most dominant new invasive in Indonesia. *P. manihoti* is originally from South America and reported to have first invaded Africa in the 1970s, causing outbreaks in large cassava areas. This pest attacks plants, especially on the cassava tip by piercing its stylet and then releasing a toxic liquid which causes the growth of the cassava tip to be stunted, the leaves to curl, wilt, and then fall. The impact of this pest attack can significantly reduce the yield of cassava tubers (Takano *et al.,* 2023). *P. manihoti* was first reported in Asia in 2008, accidentally attacking cassava in Thailand (Winotai *et al.,* 2010). Furthermore, *P. manihoti* is found in Vietnam, Cambodia, and Indonesia (Parsa *et al.,* 2012), Laos (see [http://www.vegetableipmasia.org/news/view/81\)](http://www.vegetableipmasia.org/news/view/81), and Malaysia (Dewi *et al.,* 2015). This pest is reported to cause yield losses reaching 84% in Africa, 30% in Thailand, and 30-50% in Indonesia (Winotai *et al*., 2010).

Various control efforts have been carried out, including eradicating infected plants, replacing them with relatively resistant plants, and controlling them with various chemistry insecticides. However, there is no efficient and effective control technique to tackle the invasion of this pest (Ngegba *et al.*, 2022). Likewise, with the heavy attack of *P. manihoti* on cassava in Bogor, farmers did not make control efforts intensively. The status of mealybugs is classified as an exotic and invasive pest, resulting in difficulties in proper pest control techniques.

In its home country, *P. manihoti* is not a serious problem because of the presence of local natural enemies (parasitoids) that can control these pests. *Anagyrus lopezi* (De Santis) (Hymenoptera: Encyrtidae) is a specific endoparasitoid of *P. manihoti*. This parasitoid was first introduced to Africa in 1981 and reported to be able to suppress the *P. manihoti* population by almost 90% (Gutierrez *et al.,* 1998). The introduction of *A. lopezi* from Benin, Africa to Thailand was carried out in 2010 and was followed by the release of the parasitoid on a national program (Winotai *et al.,* 2010). Furthermore, *A. lopezi* was introduced in Thailand and released in Bogor in 2014. In 2019, the distribution of this parasitoid was found in Java, Lampung, and Nusa Tenggara with parasitization rates ranging from 1.5 to 59%, especially during the dry season (Wyckhuys *et al*, 2018; Fanani *et al.,* 2019).

Biological control has several advantages, including being permanent in controlling pests, friendly, not polluting the environment, safe for humans, and not requiring a lot of costs for workers after release, when the parasitoids succeed in settling and suppressing the pest population in the field. However, there are still very limited specific studies that have been reported regarding to periodic biological control of *P. manihoti* in cassava (**Figure 1**). Detailed information regarding the use of bibliometrics is explained elsewhere (Al Husaeni & Nandiyanto, 2022; Rochman *et al*., 2024). The issues regarding this research receive quite a lot of attention, are still relevant, and provide strong enough arguments to support the novelty of this research in the future. Therefore, this study can show the important role of natural enemies in pest management, including parasitoid *A. lopezi* in controlling the cassava mealybugs, *P. manihoti*.

Figure 1. Visualization overlay based on related research trends.

2. METHODS

Monitoring the mealybug population of *P. manihoti* and their natural enemies was carried out every two weeks per month in cassava fields in Bogor consisting of three districts namely: Sukaraja, Dramaga, and Ranca Bungur during the dry season (May - September 2018) and the rainy season (October 2018 - April 2019) (n = 12 sites). Rainfall data in Bogor was obtained from the Meteorology, Climatology and Geophysics Agency, Bogor Climatology Station. The field survey protocol was carried out in several locations continuously for 12 months (Graziosi *et al.*, 2016; Le *et al.*, 2018) and focused on cassava that were planted in monoculture, had uniform age, including a part of the center of cassava production area in Bogor. A total of five linear transects were randomly selected per field, each with 10 sample plants (distance between plants 0.8 m). Sample plants in each transect start from the third row of cassava at the edge of the field.

Thus a total of 50 sample plants per field were observed concerning the abundance of mealybugs *manihoti*, ants, and predators (*P. ramburi* and *C. montrouzieri*). Observations were also made on the level of damage to plants attacked by mealybugs. The level of plants attacked by mealybugs was categorized as the proportion of plants attacked (incidence of mealybugs), the average number of *P. manihoti* per plant (abundance of mealybugs), and the level of cassava tip damage. The shoots (tip) of cassava were given a score such as score $0 =$ healthy plants, score $1 =$ curled leaves, score $2 =$ cassava tips are stunted (bunchy top), score 3 = leaves curl and stem segments shorten (distortion), score 4 = leaves fall (Fanani *et al*., 2019; Waliyudin *et al.*, 2023). Identification of mealybugs in the field is based on morphological characteristics such as coloration and the length of the wax filaments on their abdomens (Parsa *et al.*, 2012). Ants were identified using Hashimoto's identification key.

In cassava fields, *P. manihoti* was found to attack cassava tip. A total of 20 cassava shoots infested with *P. manihoti* mealybugs (bunchy top symptoms) with a length + 20 cm were randomly collected by picking in each field to assess the level of parasitization of *A. lopezi*. All samples obtained from the field were put in sample paper then stored in a cool box during the trip and taken to the Insect Bionomy and Ecology laboratory, IPB University. Upon arrival at the laboratory, the cassava tip samples were handled carefully. The number of 2nd and 3rd instar mealybug nymphs, adults, mummies with exit holes, and mummies without holes were counted and recorded. Next, the mummies were collected separately in gelatin capsules. Shoots of cassava infested with 2nd and 3rd instar nymphs and adults *P. manihoti* were replanted in wet cork media and maintained separately in transparent polyvinyl chloride plastic containers ($d = 8$ cm, $t = 11.3$ cm). All cassava tip samples infested with mealybugs were maintained in the laboratory at a temperature of $27 - 30^{\circ}$ C, relative humidity of 65 - 70%, and a daily light cycle of 12 hours of light and 12 hours of darkness and then observed every day for three to four weeks to obtained parasitoids or hyperparasitoids. Specimens were stored in Eppendorf tubes containing 70% alcohol and labeled according to the alphanumeric code of each field visited for the identification stage. Determination of hyperparasitoid identification was carried out based on Hayat (1998).

2.1. Data Analysis

All data obtained from the field were tabulated in MS. Excel consisting of the abundance of mealybugs, ants, and predators, as well as the number of plants attacked by mealybugs. The level of parasitization was calculated by dividing the number of parasitoids that appear by the number of mealybugs per sample plant, while the level of hyperparasitoids is calculated by dividing the number of hyperparasitoids that appear by the number of primary parasitoids and hyperparasitoids and then multiplying by 100 (Eq. 1). The value of the intensity of damage to cassava in each field (I) is calculated using a formula (Eq. 2) based on Townsend and Heuberger (1943).

$$
P = \frac{n}{N} \times 100\% \tag{1}
$$

where *n* is the number of mealybugs that are parasitized or hyperparasitized, and *N* is the score for all insects collected from the field.

The incidence of attacks or pest attacks is the percentage of the number of plants attacked by pests from the total sample plants observed. To calculate the incidence of pest attacks, refer to Fanani *et al.* (2019) use the following Eq. (2):

$$
I = \frac{\sum_{i=1}^{5} n_i \times \nu_i}{N \times V} \times 100\%
$$
 (2)

where *nⁱ* is the number of cassava tips with the i-th damage score, *vⁱ* is the value of the i-th cassava tip damage score, *N* is the total number of cassava tips observed, and *V* is the highest damage score value.

3. RESULTS AND DISCUSSION

3.1. Attack Incidence and Intensity of Mealybug Attacks

Overall, four species of mealybugs (pseudococcidae) were found on cassava with the composition of *P. manihoti* (50.12%), *P. marginatus* (41.26%), *Ferrisia virgata* Cockerell (5.59%), and *Psedocococcus jackbeardsleyi* Gimpel-Miller (3.02%) (n=32 951). James and Fofanah (1992) reported that the mealybugs *P. manihoti*, *P. madeirensis* (Green), and *F. virgata* infested cassava, however the incidence of attacks by *P. madeirensis* and *F. virgata* was relatively small, whereas *P. manihoti* was the most common species of mealybug in cassava. Insect observation was done regularly for 12 months in several cassava fields in Bogor showed that the highest incidence of *P. manihoti* mealybug attacks was 64% with the level of damage to plants attacked by mealybugs reaching 26% (**Figure 2**). Fluctuations in population development and attack levels of *P. manihoti* can be influenced by the rainy and dry seasons (James & Fofanah 1992; Fanani *et al.*, 2024). Carrieri *et al.* (2023) reported that the population density of insects increased during the dry season and the population significantly decreased during the rainy season.

Figure 2. Incidence of *P. manihoti* mealybug attacks and intensity of cassava damage.

The high incidence of cassava attacks and damage occurred during the dry season in July 2018 with an average daily rainfall of only 73 mm per month. In contrast, from August 2018 to April 2019, the incidence rate of *P. manihoti* attacks and plant damage tended to decrease significantly. This decrease occurred when entering the rainy season with rainfall tending to increase from October 2018 (rainfall of 466 mm) to April 2019 (rainfall of 631 mm) (**Figure 3**). Rainfall is one of the key determinants of *P. manihoti* population dynamics, as in dry areas and seasons it can trigger outbreaks in the field (Gutierrez *et al.*, 1988). The lowest level of *P. manihoti* attacks occurred during the rainy season with a high rainfall intensity of 429-636 mm/month. The presence of rainfall on cassava can cause mealybugs to fall and cause them to move mechanically, causing death and having an impact on reducing the population of mealybugs. The low population and level of mealybug attacks can indirectly reduce the occurrence of plant damage.

3.2. Population Dynamics of Mealybug *P. manihoti*

The average number of each life stage of *P. manihoti* was higher in the dry season in July-August 2018 than in other months. 2nd instar, 3rd instar, and adult P. manihoti nymphs per cassava tip were mostly found in July 2018 with an abundance of around 1-2 individuals each (**Figure 4**). The highest number of ovisacs and perforated mummies were found in July 2018 with a low abundance, while the most non-perforated mummies were found in August 2018 with an abundance of around 2 mummies per cassava tip. As the rainy season enters in October 2018, the mealybug population tends to decrease significantly. In general, very low populations of mealybugs in the 2nd instar, 3rd instar, and adult were found in February

2019, while the number of mealybugs forming mummies was still higher. The low population in each phase of mealybug development is followed by a high number of mealybugs becoming mummified. At the same time, the level of parasitization of *A. lopezi* was higher than that of mealybug populations on cassava (**Figure 3**). This shows that the presence of introduced parasitoids has a real impact on the low population of mealybugs in the field. This is in line with the study of Roltsch *et al.* (2006) showed that the lower mealybug population can indirectly reduce the level of parasitization.

Figure 4. Dynamics of each life stage *P. manihoti*.

3.3. Parasitization Dynamics of *P. manihoti* **by** *A. lopezi*

This study illustrates the success of *A. lopezi* in suppressing the population of *P. manihoti* to remain low in the field (**Figure 5**). During the dry season in May 2018, the average population of *P. manihoti* found was around 8 individuals per cassava tip with the parasitization level of *A. lopezi* reaching 16%, ± 6.7. The mealybug population increased to its peak in August 2018 with the number of mealybugs around 17 per cassava tip and the parasitization level of *A. lopezi* reaching 46% + 7.3. The mealybug population of *P. manihoti* decreased significantly from September 2018 to April 2019. It was followed by the parasitization level of *A. lopezi* which tended to decrease further. The lowest mealybug population was found during the rainy season in November, December 2018, and February 2019 with an average population of 1-2 mealybugs per cassava tip and a level of *A. lopezi* parasitization ranging from 12 to 36%. Hammond and Neuenschwander (1990) reported that in Nigeria, *A. lopezi* was able to control the *P. manihoti* population significantly and sustainably until the mealybug population remained low, ranging from 1 to 10 individuals per cassava tip with parasitization levels ranging from 24.6 to 25.7%. The level of parasitization is considered to increase with increasing the mealybug population (Fanani *et al.,* 2020a; Waliyudin *et al.,* 2023; Fanani *et al.,* 2023; Fanani *et al.,* 2024).

In 2014, *A. lopezi* is a newly introduced parasitoid in Indonesia and was released in Bogor (Wyckhuys, 2014). After 3-4 years, this parasitoid can still be found in several provinces in Indonesia with parasitization rates reaching 1.5-59% (Fanani *et al.,* 2019), while the parasitization rate of *A. lopezi* in Vietnam is around 49.9-52.1% on average (Le *et al.,* 2018). Some examples of successful biological control of mealybugs are the control of cassava mealybugs, *P. manihoti* in Africa (Neuenschwander, 2001); mango mealybug, *Rastrococcus* invaders (Williams) in West Africa (Pitan *et al.,* 2000); hibiscus mealybug, *M. hirsutus* in the Caribbean (Kairo *et al.* 2000); and the papaya mealybug, *P. marginatus* in Guam (Meyerdirk *et al.,* 2004). The classic biological control that has been successfully carried out in Indonesia is the control of *Plutella maculipennis* Curt. (Lepidoptera: Plutellidae) by the parasitoid *Diadegma semiclausum* Hellen (Hymenoptera; Ichneumonidae) (Vos 1953). Furthermore, the parasitoid *A. lopezi* is successfully control *P. manihoti* in several sites of Indonesia (Fanani *et al.,* 2019).

Figure 5. Parasitization fluctuation of *A. lopezi* on *P. manihoti*.

3.4. Hiperparasitism

The presence of hyperparasitoid species originating from *P. manihoti* mummies was discovered from June 2018 to January 2019, with a hyperparasitization rate against *A. lopezi* of around 2.39% per month and the highest hyperparasitization rate was around 6% in July 2018 (**Figure 6**). The hyperparasitoid species that attack *A. lopezi* are dominated by *Prochiloneurus* sp. and followed by *Chartocerus* sp. In August, November, and December 2018, *Prochiloneurus* sp. was most commonly found as indicated by the high percentage of mummies obtained from *P. manihoti* samples. Two hymenopteran species emerged from mummified mealybugs *P. manihoti* were collected from cassava fields. The two hyperparasitoid species that were identified were *Chartocerus* sp. (Hymenoptera: Signiphoridae) and *Prochiloneurus* sp. (Hymenoptera: Encyrtidae) which is the primary hyperparasitoid of *A. lopezi* and *A. papayae*. Neuenschwander and Hammond (1988) reported that *Chartocerus* spp. and *Prochiloneurus* spp. are the two hyperparasitoid species most often found attacking *A. lopezi*. In samples collected from *P. manihoti* mummies, the percentage of hyperparasitoids attacking *A. lopezi* began to decrease after July 2018 (**Figure 6**). The low population of mummies collected from the field can be an indication to evaluate the cause of the low percentage of hyperparasitization and also because the abundance of mealybug populations in the field is relatively low (**Figures 5** and **6**). In Vietnam, three hyperparasitoid species of *A. lopezi* have been reported: *Chartocerus* sp. near walker Hayat, *Prochiloneurus* sp., and *Promuscidae unfasciativentris Girault* (Hymenoptera: Eriaporidae) with hyperparasitization levels ranging from 2.79 to 5.38% (Le *et al.* 2018). *Prochiloneurus insolitus* (Alam) and *Chartocerus* hyalipennis (Hayat) were found in association with *A. lopezi* in Africa.

In Southern California, two hyperparasitoids were found, namely *Chartocerus* sp. and Marietta sp. with a very low level of hyperparasitization. Hyperparasitoids appeared in 5 of the 286 mummies collected (1.7%) (Roltsch *et al.,* 2006). Fischer (1991) reported that hyperparasitization of *A. lopezi* in Togo ranged from 20 to 90%. Even though the level of hyperparasitization is high, this does not pose a serious threat to the efficiency and effectiveness of the parasitoid *A. lopezi* in controlling *P. manihoti* (Neuenschwander & Hammond, 1988; Fischer, 1991). The experimental results of Goergen and Neuenschwander (1992) showed that increasing the number of hyperparasitoids had no real impact on reducing the efficiency and effectiveness of *A. lopezi*. During the rainy season, especially in February - April 2019, the mealybug population in the field was very low. Thus, it could be an influencing factor in the low or even non-discovery of hyperparasitoid species. A similar thing happened on cassava in Nigeria, with the lower density of *A. lopezi* causing the percentage of hyperparasitization to decrease significantly (Neuenschwander & Hammond, 1988).

The periodic decline in the level of attacks by the hyperparasitoid *Marietta* sp. is influenced by the dynamics of the decline in the population of the parasitoid *A. kamali*, which is its host (Roltsch *et al.,* 2006). In Vietnam, three hyperparasitoid species were reported, *Chartocerus* sp. near walker Hayat, *Prochiloneurus* sp., and *Promuscidae unfasciativentris* Girault (Hymenoptera: Eriaporidae) with hyperparasitization levels ranging from 2.79 to 5.38% (Le *et al.,* 2018). *Prochiloneurus insolitus* (Alam) and *Chartocerus hyalipennis* (Hayat) were found in association with *A. lopezi* in Africa (Plata *et al.,* 2023). In Southern California, two hyperparasitoids were found, namely *Chartocerus* sp. and *Marietta* sp. with very low levels of hyperparasitization (Shivakumara *et al.,* 2022).

Figure 6. Percentage of hyperparasitization in *A. lopezi*. The number of parasitoids and hyperparasitoids is shown at the top of the bar chart.

3.5. Abundance of Ants on Cassava

During field observations, the average number of ants associated with cassava was around 1-5 per plant. The lowest ant population was found in June and November 2018, while the highest ant population was found in January 2019 (**Figure 7**). Fanani *et al.* (2019) reported that the presence of ants associated with cassava can increase the possibility of damage to plants attacked by *P. manihoti*. Cudjoe *et al.* (1993) showed that several ant species could reduce the level of parasitization of *A. lopezi* on *P. manihoti*. Mealybugs secrete honeydew as a food source for ants. On the other hand, the presence of ants can protect mealybugs from natural enemies such as predators and parasitoids (Helms & Vinson, 2008), as a result, the presence of ants can indirectly reduce the level of parasitization of mealybugs (Cudjoe *et al.* 1993; Mansour *et al.*, 2011).

The most dominant ant species found on cassava attacked by mealybugs was *Anoplolepis gracilipes* Smith (53.25%) followed by *Tapinoma melanocephalum* Fabricius (36.14%) and other ant species (10.60%) consisting of: *D. thoracicus, Technomyrmex albipes* Smith, *Cremetogaster* sp., *Camponotus* sp., *Polyrachis* sp., *Odontoponera* sp., *Diacamma* sp., and *Pheidole* sp. (n = 7176) (**Figure 8**). Cudjoe *et al.* (1993) and Fanani *et al.* (2019) found several species of ants that can associate with mealybugs on cassava, namely *A. gracilipes, Camponotus acvapimensis Mayr, Pheidole megacephala* (Fabricius), *Crematogaster luctans* Forel, *Acantholepis capensis* Mayr, *Monomorium* sp., *Oecophylla longinoda* (Latreille), *Dolichoderus thoracicus* Smith*, T. melanocephalum* and *Paratrechina* sp. Mealybugs associate with other insects, in this case, they can have a mutualistic symbiotic relationship with ants.

Figure 7. Population dynamics of ants on cassava.

Figure 8. Proportion of ant species population on cassava.

These mealybugs secrete honeydew which is loved by ants. Apart from helping to spread mealybugs, ants also protect these mealybugs from predatory beetles, parasitoids, and other natural enemies. Ants also protect papaya mealybug colonies by cleaning detritus from honeydew secretions released by papaya mealybugs which may be harmful to the colony. Mansour *et al.* (2011) reported that the strong mutualistic relationship between mealybugs and ants can be one of the defense systems for mealybugs from natural enemies in the form of predatory insects and parasitoids. Thus, the presence of ants can indirectly provide a protective service for mealybugs to survive. In addition, several studies show that the presence of ants can influence the population dynamics of mealybugs, aphids and other honeydew-producing hemipteran insects (Katayama & Suzuki 2002). However, attendance of the ant population can decrease the effectiveness of parasitoid insects (Fanani *et al.,* 2020b). Therefore, a high abundance of ant populations can indirectly increase the population of mealybugs which in turn can result in serious damage to plants.

3.6. Predator Insect

The most dominant mealybug predator insects found on cassava are *P. ramburi* and *C. montrouzieri*. However, the population is always low because this may be due to the low presence of mealybugs which are their prey. From May 2018 to April 2019, the populations of these two predators were relatively low (**Figures 9A** and **9B**). Among all the development phases of the two predators, only *P. ramburi* eggs are often found in the field even though the population is relatively low, ranging from 1 to 2 eggs per plant, especially in May-September 2018 and March 2019.

Figure 9. Abundance of predator insects: *P. ramburi* (A) dan *C. montrouzieri* (B).

C. montrouzieri eggs were not observed because their size is very small so they are difficult to find on cassava. The presence and increase in the predator population of *P. ramburi* are usually quite late when compared with the development of the whitefly population of *P. manihoti* on cassava. Therefore, the presence of natural enemies (predators) is greatly influenced by the availability of prey (Landis *et al.*, 2000). Even though these two predators are generalists, the average rainfall with relatively high intensity in the Bogor area can indirectly kill and reduce the population of several insect pests such as mealybugs, aphids, and mites which have the potential to be prey for predators. Thus, they can reduce the presence of predators or even cause the absence of these natural enemies (Zia *et al.,* 2008).

The presence of the predators *P. ramburi* and *C. montrouzieri* can be exploited partially, namely if the main natural enemy (parasitoid) is not capable enough to keep the mealybug population low. Especially during the dry season, if the population of mealybugs and the level of attack on plants is high, mass release of predators might be a quick and appropriate alternative to control mealybug attacks on cassava fields. *P. ramburi* is quite effective in controlling *P. manihoti* because it has a type II functional response characteristic, which means that as the prey population increases, the rate of predation will decrease and maximum prey mortality occurs when the prey population is low. During its development, each *P. ramburi* larva was able to prey on as many as 750 mealybugs. Another predator that is also often found on cassava is *C. montrouzieri*. During its life development, individual *C. montrouzieri* larvae were able to prey on 844 1st instar nymphs of *P. marginatus*. The adult is capable of preying on up to 2000 nymphs-1 of *P. marginatus*. Apart from that, this predator preys on the eggs stored in the ovisac (egg sac) of the whitefly *P. marginatus*, amounting to 68-190 eggs. Biological control of cassava mealybugs has great potential to be developed because pest control using parasitoids as natural enemies of mealybugs provides a sustainable, timeless impact on reducing pest populations and does not require expensive costs.

4. CONCLUSION

P. manihoti was the most abundant attack on cassava, especially during the dry season. The dynamics population of mealybugs and attacks are influenced by rainfall. Parasitoid *A. lopezi* was able to suppress the populations of the mealybugs sustainably with parasitization rates reaching 46 and 15%, respectively. The hyperparasitoids found were *Chartocerus* sp. and *Prochiloneurus* sp. However, hyperparasitization was considerably lower during the year. The most dominant ant found was *A. gracilipes*. Predators *P. ramburi* and *C. montrouzieri* have very low populations in the field. The efforts to monitor the development of the mealybug populations and the level of parasitization on a wider area are needed to determine the effectiveness of parasitoid in suppressing mealybug populations.

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6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. The authors confirmed that the paper was free of plagiarism.

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