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Range of host plants, spatial distribution, and insect predator of *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) as an emerging pest of cassava plants in Bali, Indonesia

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Abstract. Supartha IW, Widaningsih D, Susila IW, Yudha IKW, Utama IWEK, Wiradana PA. 2022. Range of host plants, spatial distribution, and insect predator of *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) as an emerging pest of cassava plants in Bali. *Biodiversitas* 23: 3022-3030. The cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) is a very destructive pest in cassava farming in Indonesia. The insect has already made its way into Indonesia, where it has attacked cassava plants with indications of a bunchy top, which may cause plant death. The objectives of this study were to 1) discover and identify an invasive pest of cassava mealybug pest, *P. manihoti*, that attacks cassava plants in Bali; 2) map the distribution and abundance of these pest species populations on cassava plants in Bali; 3) describe the structure of the pest population in cassava plantations in Bali; 4) identify the dominant factors that influence the population structure of *P. manihoti* in Bali, and 5) evaluate the predator fauna in regulating the population. The findings revealed that the mealybug species that attacked cassava in Bali was *P. manihoti*, an exotic species that had never been published earlier. With a high population abundance, the pest has spread far and effectively overall in regencies/cities in Bali Province. In all regency/city regions, the population structure of *P. manihoti* was dominated by age groups of instar-1 and two nymphs, with the exception of the Buleleng Regency, which was dominated by age groups of nymphs instar 3 and 4. The nature of polyphagy and cosmopolite features that are readily adaptable to the environment and host plant variables, farming pattern, temperature and rainfall factors were the key factors impacting the population structure of *P. manihoti* in the field. *Cryptolaemus montrouzieri*, *Chrysoperla carnea*, and *Scymnus* sp. were discovered to have a role in managing *P. manihoti* populations in the field. The predator fauna community is structurally diverse, abundant, and has a low dominance. *Cryptolaemus montrouzieri* was the most dominant of the three producer species in the field. These results indicate that there is a positive potential shown by predatory mealybugs in the field and can be used as an initial model by related parties in formulating policies related to controlling insect pests of cassava plants.

Keywords: Biocontrol, biological parameters, insect predator, integrated pest management, invasive pest

INTRODUCTION

Indonesia is the fourth largest cassava (*Manihot esculenta*) producer in the world after Brazil, Nigeria and Thailand (Supatmi 2017; Yi et al. 2019). Cassava production in Indonesia has fluctuated, which decreased from 2010 to 2015 (BPS 2018). One of the factors that caused the fluctuation and decline in production was the disturbance of pests and diseases (Velásquez et al. 2018; Armengot et al. 2020; Skendžić et al. 2021). Several types of diseases that attack cassava in Asia are bacterial blight or cassava bacterial blight (CBB) caused by *Xanthomonas campestris* pv. *manihoti*, cassava mosaic disease (CMD) virus, and cassava brown streak disease (CBSD) (Amer 2017). Meanwhile, the main types of pests, which being the problem in cassava cultivation in Asia are whiteflies (*Bemisia tabaci*), mealybugs (*Phenacoccus manihoti*) and red spider mites (*Tetranychus urticae*) (Parsa et al. 2012; Graziosi et al. 2018). The presence of mealybug as an important pest on cassava is further reported to damage

cassava in several African and South American countries (Adegbite et al. 2021; Eni et al. 2021). The mealybug *Phenacoccus* sp. (Hemiptera: Pseudococcidae) is the most frequent insect that causes damage to cassava in both countries (Sartiami et al. 2015).

Phenacoccus manihoti Matile-Ferrero is the most harmful pest on cassava in many countries in the world (Yonow et al. 2017; Joshi et al. 2020; Venkatesan et al. 2021). This pest can damage plants directly by sucking the fluids and poisoning the plants with their saliva (Bellotti et al. 1999; Barilli et al. 2014). As a result, the cassava develops bunchy top of the leaves and this can result in the death of the plants (Maruthi et al. 2019; Shirima et al. 2019). This insect is an invasive pest that has entered Indonesia and spread in several areas, especially in Bali Province (Supartha et al. 2020). The entry of this pest into Bali province is strongly suspected through the distribution of ornamental plants from other provinces or abroad (Hamid et al. 2018; Supartha et al. 2022). The observational results show that a similar symptom has been

founded in several cassava cultivation sites in Bali. There is a strong indication that the symptom of bunchy top on the cassava is caused by the cassava mealybug *P. manihoti*.

This type of pest was not previously known by cassava farmers in Bali. But the farmers thought that the mealybug was the same as the type of mealybug they knew before. Therefore, most farmers control it by using synthetic insecticides, which are commonly used for pest types found in other crop commodities (Tembo et al. 2018; Yan et al. 2018; El-Bakry et al. 2021; Lin et al. 2021). Although these control efforts have been carried out, the condition of the mealybug population in the field is still high. For this reason, a special approach is needed to deal with these pests in accordance with the principles of integrated pest control (IPM), especially control components originating from the ecosystem (Deguine et al. 2021; Yuliadhi et al. 2021). One of these components is the use of natural enemies from both predators and parasitoids (de Pedro et al. 2021; Chou et al. 2022; Mitchell et al. 2022; Rubio et al. 2022). Natural enemies from the predatory group have several advantages, including: a) being able to prey quickly; b) being able to kill various stages of prey and c) being able to take large amounts of prey (Yuliadhi et al. 2021). The use of natural enemies in controlling mealybugs and other harmful pests has been pioneered previously (Wyckhuys et al. 2018). The use of natural enemies in controlling mealybugs in cassava has been successfully carried out in 25 countries, such as in Africa and Thailand, with beetles *Plesiochrysa ramburi* and Coccinellidae as predators of mealybugs in these countries (Supartha et al. 2020).

Based on these findings, the study's aims include finding and evaluating the abundance and distribution of *P. manihoti* in cassava crops, as well as natural enemies that may be utilized as biocontrol agents for this pest in the future. In this research, environmental parameters that influence the population structure and community of predatory insects related to different cassava cultivars in Bali Province were also measured.

MATERIALS AND METHODS

Study area

This research was conducted on a field and laboratory scale. Field research was carried out in all regencies/cities in Bali Province, Indonesia, which conduct cassava cultivation. Meanwhile, laboratory research which includes specimen maintenance, pest identification, and pest status determination, was carried out at the Integrated Pest Management Laboratory (IPMLab), Faculty of Agriculture, Udayana University. The research was conducted from March to July 2019.

Sample site

The sampling locations were conducted in all regencies/cities in Bali Province, namely in Badung Regency (8°39'24" S 115°14'59" E); Bangli (8°11'52" S 115°25'51" E); Buleleng (8°9'42" S 115°2'45" E); Gianyar (8°28'56" S 115°18'725" E); Jembrana (8°23'20" S

114°44'32" E); Karangasem (8°28'0" S 115°37'12" E); Klungkung (8°30'21" S 115°22'46" E); Tabanan (8°28'59" S 115°5'52" E) and Denpasar City (8°39'24" S 115°14'59" E). Leaf and shoot samples were taken purposively as many as 5 infected cassava leaves/locations according to the availability of host plants in the field. Leaf samples from each location were temporarily stored in a clear plastic bag measuring 2 kg, then stored in a cool box and then brought to the laboratory. Each leaf sample consisted of 5 leaves was then observed in the laboratory for the number of species and population counts of predators and prey associated with cassava in the field.

Phenacoccus manihoti

Inventory and identification of Phenacoccus manihoti

Nymphs and adults of *P. manihoti* were counted and recorded and then stored in a collection bottle filled with 96% alcohol to be stored in the refrigerator for inventory and morphological identification purposes, each of which was given a location label and a description of the host and collector. Identification of *P. manihoti* was carried out by matching the specimen with the morphological characteristics of *P. manihoti*, which corresponded to the key of determination (Parsa et al. 2012; Sartiami et al. 2015).

Abundance and distribution of Phenacoccus manihoti

To determine the abundance of mealybugs in each district, it was done by counting the population of the mealybug *P. manihoti* from instars 1, 2, 3 and adult, then presented in graphical form. Meanwhile, the distribution of the population was calculated by recording the population at each coordinate point of the location where *P. manihoti* was taken in the field to be depicted in the form of a map of the distribution of the pest population.

Data analysis

To determine the diversity, abundance, predator dominance at each location and, host plants were analyzed by the following formula:

Measurement of diversity index used an index developed by Shannon and Wiener (H') (McCarthy and Magurran 2004) through the following equation:

$$H' = -\sum p_i \cdot \ln \cdot p_i$$

$$p_i = \frac{n_i}{N}$$

Where, H': Shannon Wiener Index; n_i: Number of individuals for the species observed; N: Total number of individuals.

The diversity index was grouped into three categories, namely: if H' < 1.5 the diversity is low; H' = 1.5-3.5 the diversity is moderate, and the value of H' > 3.5, the diversity is high.

Measurement of abundance index used the Margalef index (McCarthy and Magurran 2004):

$$R1 = \frac{S-1}{\ln N}$$

Where, R1: Abundance index; S: Number of species found; ln: Nature logarithm; N: Total number of individuals.

Values, R1<3.5: low; R1>3.5-<5.0: moderate; R1>5.0: high.

Measurement of dominance index used the Menhinick index (McCarthy and Magurran 2004):

$$D = \sum \left(\frac{ni (ni-1)}{N (N-1)} \right)$$

Where, D: Dominance index; N: Total number of individuals; ni: Number of i-species individuals.

Values, D≤0.00-0.30: low; D>0.30-0.60: moderate; D≥0.60-1.00: high.

RESULTS AND DISCUSSION

Identification of the mealybug *Phenacoccus manihoti* which attacks the cassava in Bali

The mature internode is obviously covered with a white waxy substance morphologically (Figure 1A). The cassava mealybug *P. manihoti* body is divided into 3 parts: the head, thoracic, and abdominal. A pair of antennae with 9 segments on adults and 6 segments on early instar nymphs and a pair of rather enlarged eyes are positioned on the head (Illathur et al. 2018). Furthermore, there are several quinquelocular poruses affecting the ventral surface of the

head (Figure 1D), as mentioned by Parsa et al. (2012) that the mesothorax is the largest part of the body with three pairs of limbs of the same size. These ticks always develop into female adults and only reproduce parthenogenetically (Parsa et al. 2012; Fanani et al. 2020).

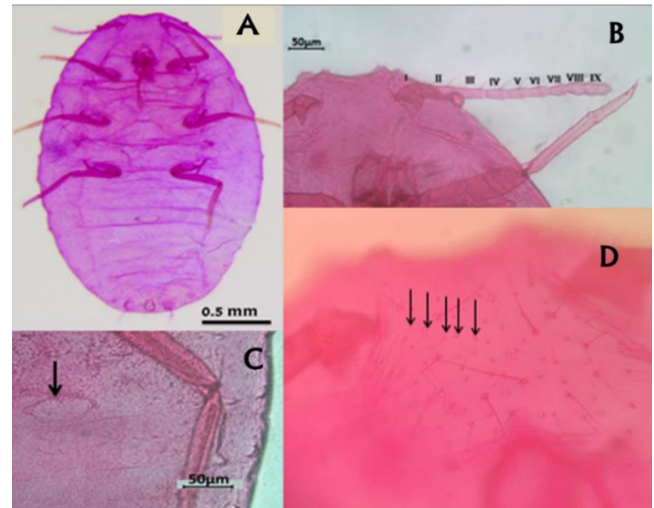


Figure 1. Morphological characteristics of female *Phenacoccus manihoti*. The whole body of *P. manihoti* (A); Antennae (B); Circulus (C); Quinquelocular shaft (D)

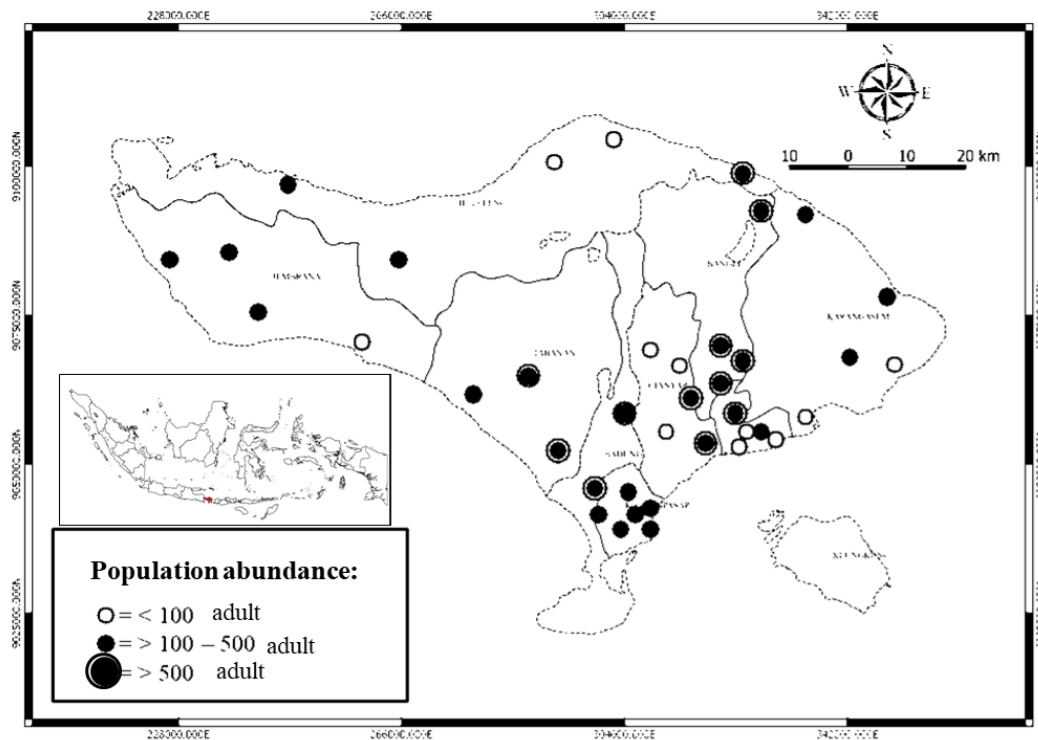


Figure 2. The distribution map of *Phenacoccus manihoti* in Bali Province, Indonesia

Distribution and abundance of mealybug population in Bali Province

The findings of the field study revealed that the *P. manihoti* population was distributed on the surface over the cassava farming regions of Bali. The presence of cassava mealybug was discovered at all survey sites in Bali's nine regencies/cities. The population distribution in each regency/city in Bali was generally high, ranging from 100 to >500 adults per plant (Figure 2). The presence of *P. manihoti* colonies on plant stems consists of pink nymphs, adult females, and white egg sacs (*ovisacs*) similar to cotton, as previously described by Weintraub et al. (2017) that the shoots of the plant carrying the cassava mealybug colony would look wound and clumped (*bunchytop*). Furthermore, this cassava mealybug may produce symptoms such as shorter and curved stems, dropped leaves, and irregular growth (Parsa et al. 2012).

The cassava mealybug, *P. manihoti*, which has been identified in every regency/city in Bali, has migrated from the lowlands to the highlands. This cassava mealybug affected almost all cassava in lowland locations, such as Denpasar, which is 10 meters above sea level (masl). Meanwhile, communities at greater elevations (>500 masl), such as Kintamani District in Bangli Regency, which has a height of 532 masl, saw a similar phenomenon. The findings of this study support the survey results of earlier research by Weintraub et al. (2017) that the spread of *P. manihoti* on the island of Java is based on cassava at altitudes ranging from 15 meters above sea level (Kranjan Village, Pacitan Regency) to 840 (masl) (Semplak Village, Sukabumi Regency). The existence of mealybug colonies and symptoms of bunchy top attacks on cassava produced by *P. manihoti* nymphs and adults revealed the prevalence of mealybug in all districts and cities in Bali. According to Abduchalek et al. (2017) the presence of this mealybug on plants is also indicated by the appearance of white ovisac-like cotton, particularly on the damaged cassava shoots.

The presence of biotic variables, such as the availability and distribution of host plants grown by farmers (Yonow et al. 2017; Wahyuni et al. 2017; Tong et al. 2019), has a substantial effect on the spread of cassava mealybug in all regencies in Bali. There are a lot of things that can help the population of *P. manihoti* grow, such as cassava (Souissi and Le Rü 1998). Fortunately, our field data indicates that, in addition to damaging cassava crops, *P. manihoti* may also affect other agricultural crops. More research is needed to confirm *P. manihoti* potential invasion of additional host plant families, which might then be used as an early warning system for concerned stakeholders and farmers (Furlan et al. 2021; Sharafi et al. 2021). Biotic variables like temperature and rainfall, in addition to host plant conditions, influence the dispersal of *P. manihoti* in the field (Alonso et al. 2021). Ideal temperature on 28°C conditions and low rainfall can trigger the development of the mealybug population more rapidly (Walton et al. 2004).

The population abundance of *P. manihoti* on cassava varied by regency/city in Bali Province. Figure 3 illustrates that Bangli Regency had the largest population abundance (7336 adults), followed by Badung (4535 adults) and Gianyar (4203 adults). Meanwhile, the Karangasem and

Jembrana Regencies had the lowest population abundance, with 612 and 716 adults, respectively. The average population abundance of *P. manihoti* in Bangli Regency was >100-500 adults, whereas in Badung and Gianyar Regencies, it was >100-500 adults, respectively. Meanwhile, the population abundance ranged from 100-500 adults in the Karangasem and Jembrana Regencies, respectively. The existence of these differences in abundance could be predicted because each district, which is made up of villages and sub-districts, has different ecosystem characteristics in the form of variations in 1) altitude, which affects temperature, humidity, and rainfall; 2) cropping characteristics such as types of varieties, planting area, and cropping patterns (monocultures and polyculture); 3) technical cultivation; and 4) crop availability (Dirgayana et al. 2021).

Population structure of *Phenacoccus manihoti* on cassava cultivation in Bali Province

In Bali, the population pattern of the mealybug *P. manihoti* on cassava varied by regency/city. However, population structure, as determined by age structure and population density, suggested that the population that spreads in each regency/city in Bali was dominated by instar-1 and 2, with the exception of Buleleng Regency, which was dominated by instar 3 and 4 (Figure 4). Figure 4 illustrates that the number of eggs selected in plants was a reproductive feature of the parthenogenetic cassava mealybug *P. manihoti*, which means that females produce all offspring without the involvement of males in their reproductive system (Parsa et al. 2012). The adult cassava mealybug, *P. manihoti*, may lay 500 eggs in the ovisac, or egg sac (Wardani et al. 2014).

Based on the number of egg groups counted in the field, from instar-1 nymphs to instar-4 nymphs of *P. manihoti*, egg groups were created in the range of 29-454 on 25 cassava leaves. *P. manihoti* cassava mealybug nymphs have been found to have rather high individual totals, ranging from 46 to 380 adult/25 cassava leaves. The adult population density varied from 24.48 to 293.48 per leaf. The Bangli Regency region had the greatest population density (293.44 adults/leaf), while the Karangasem Regency area had the lowest (24.48 adults/leaf) (Table 5).

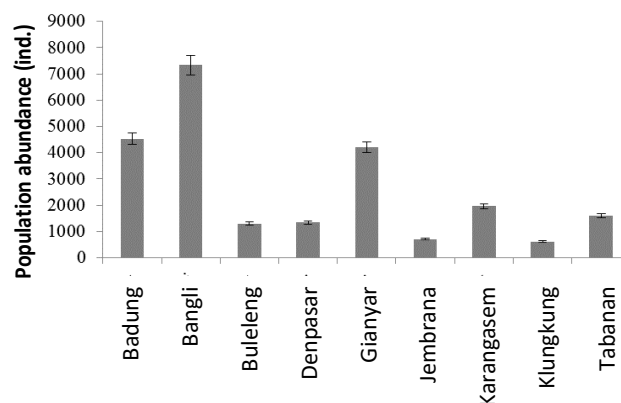


Figure 3. Population abundance of *Phenacoccus manihoti* on cassava crop in Bali Province, Indonesia

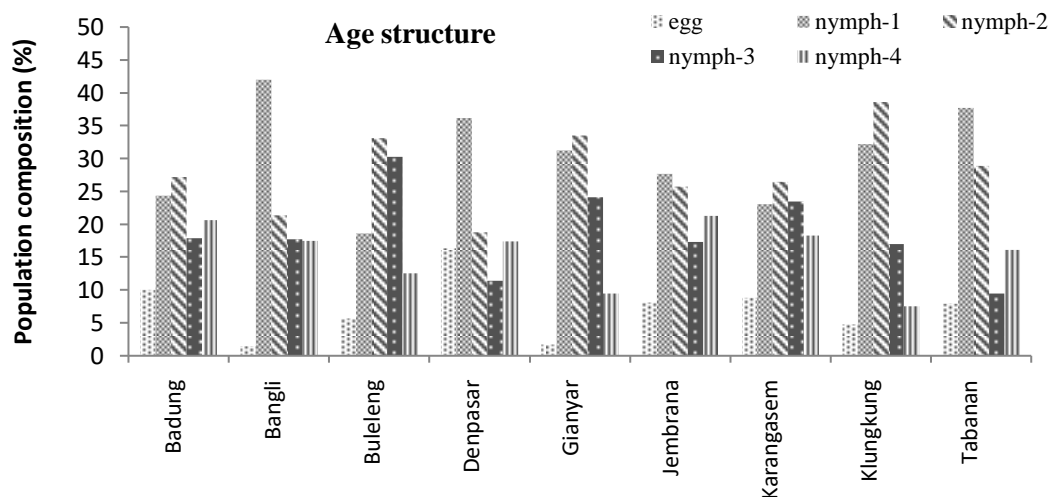


Figure 4. The structure population of *Phenacoccus manihoti* on cassava crop in Bali Province, Indonesia

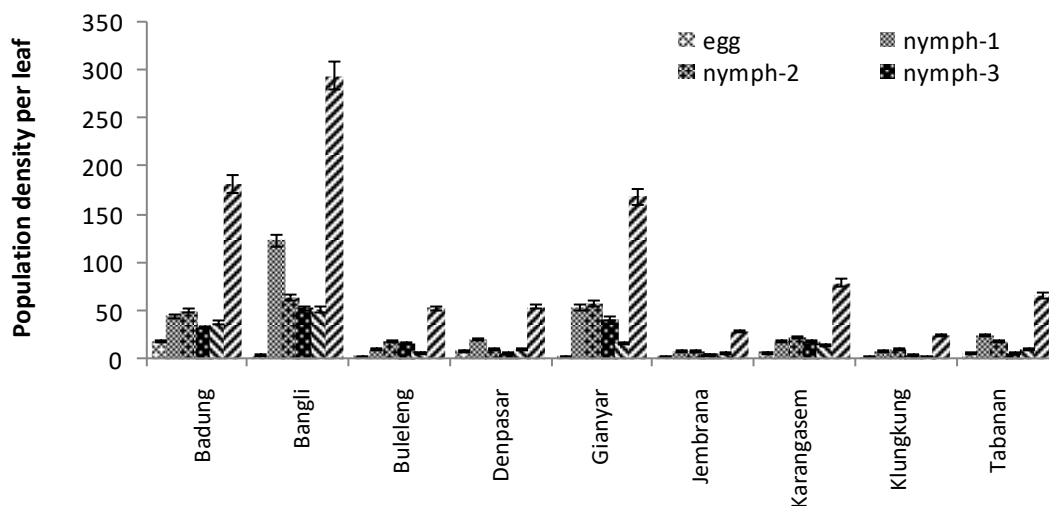


Figure 5. Population density of *Phenacoccus manihoti* per shoot on cassava crop in Bali, Indonesia

Factors affecting the abundance of *Phenacoccus manihoti* population in Bali Province

The findings revealed that various parameters influencing the *P. manihoti* population in the field were directly controlled by the availability of the host plant and the farmers' crop production strategy. This pest's capacity to adapt to the availability of host plant species in the field. *P. manihoti* was discovered in the field in three cassava varieties: UJ-5, Adira-1, and Malang-6. *Phenacoccus manihoti* attacked the three types in the field, although the presence of these three varieties was not detected in all districts/cities in Bali. Such as the Adira 1 variety, which was not identified in Denpasar City, and the Malang 6 variety, which was identified in Gianyar, Jembrana, Klungkung, and Tabanan Regency (Table 1). The cultivars UJ5, Adira 1, and Malang 6 are identified as having a high cyanide content. The intrinsic indication of *P. manihoti*, namely that the pre-maturity phase of growth is shorter and its superiority is greater, indicates that the life of *P.*

manihoti on cassava varieties containing high cyanide acid is better utilized by *P. manihoti* (Wardani et al. 2014).

In addition to the availability of cassava varieties in the field, the population of *P. manihoti* may be impacted by external variables such as farmer cropping practices. *Phenacoccus manihoti* populations were greater in cassava crops cultivated in a monoculture cropping system (Table 1). *Phenacoccus manihoti* has a high population density in all the regencies/cities that implement monoculture farming, although intercropping patterns have only been discovered in Badung, Buleleng, and Karangasem Regencies. The population of *P. manihoti* in the intercropping pattern tended to be smaller, which is due to the impact of the canopy of intercropping plants, which suppresses *P. manihoti* development, which is not optimum in the micro-cropping environment. The temperature factor has an impact on the cassava mealybug's ability to survive at an optimum temperature of 28°C. This perfect temperature condition causes the population of cassava mealybugs to achieve its best fitness (Walton et al. 2004).

Environmental physical factors such as rainfall, temperature, wind speed and humidity also affect the population structure of *P. manihoti* as shown in Table 2. agro-climatic data during field sampling from December 2018 to July 2019. Rainfall, temperature and humidity, with the average values of 32.4-711.2 mm; 26-31°C and 73-81% during the field study affected the abundance of the field mealybug population. Wind speed with an average value of 7.5-22.8 km per hour could help in the spread of *P. manihoti* in the field. Rainfall between 140 mm-278.4 mm could reduce the mealybug population. Mealybugs are able to live at an air temperature of 14.7°C and develop optimally at a temperature of 28°C.

The type of insect predator fauna associated with *Phenacoccus manihoti* in cassava in Bali Province

Field observations showed the existence of three predator species in the cassava crop, namely *Cryptolaemus montrouzieri*, *Chrysoperla carnea*, and *Scymnus* sp. (Figure 6). *Scymnus* sp. and *C. montrouzieri* are two significant species that prey on mealybugs, according to Kairo (2013). Which according to findings of this research, the most common predator species discovered in cassava in Bali were *C. montrouzieri* (156 adult), *C. carnea* (35 adult), and *Scymnus* sp. (17 adult) (Table 3).

Table 1. Characteristics of extrinsic factors influencing *Phenacoccus manihoti* population on cassava cultivation in Bali Province, Indonesia

District/City	Population density (adult)					Altitude (masl**)
	Cassava varieties			Cropping pattern		
	UJ 5	Adira 1	Malang 6	Monoculture	Intercropping*	
Badung	3505	815	215	2905	1630	29-973
Bangli	2613	1273	1087	4973	-	237-927
Buleleng	401	419	454	1241	63	17-375
Denpasar City	1357	-	-	1357	-	10-84
Gianyar	795	3617	-	4412	-	52-503
Jembrana	440	276	-	716	-	08-38
Karangasem	134	138	341	439	173	11-343
Klungkung	1779	17	-	1796	-	20-323
Tabanan	761	853	-	1614	-	51-732

Note: * Intercropping: multiple cropping with banana and coconut. **masl: meters above sea level.

Table 2. The data of weather in Bali Province, Indonesia

Regency/City	Rainfall (mm)	Temperature (°C)	Humidity (%)	Wind Velocity (KmPh)
Bangli	39.6-143.7	28-30	73-79	7.5-9.1
Gianyar	39.6-143.7	28-30	73-79	7.5-9.1
Klungkung	198.9-480.5	29-30	77-80	7.7 11.7
Denpasar	32.4-83.5	28-29	73-77	17.1-22.8
Badung	39.6-143.7	28-30	73-79	7.5-9.1
Tabanan	39.6-143.7	28-30	73-79	7.5-9.1
Negara	69.6-214	30-31	74-79	8.7-11
Buleleng	565.6-711.2	26-27	79-81	7.7-10.9
Karangasem	198-480.5	29-30	77-80	7.7-11.7

Table 3. The Structure of predator communities associated with cassava mealybug *Phenacoccus manihoti* in Bali Province, Indonesia

Predator Species	Badung	Bangli	Buleleng	Denpasar	Gianyar	Jembrana	Klungkung	Karangasem	Tabanan
	Total Individuals (adult)								
<i>C. mentrouzieri</i>	30	14	15	7	28	20	17	18	29
<i>C. carnea</i>	10	5	4	7	3	3	1	3	5
<i>Scymnus</i> sp.	0	2	6	0	3	0	2	4	0
N Predator	40	21	25	14	34	23	20	25	34
R1	0.54	0.66	0.62	0.76	0.57	0.64	0.67	0.62	0.57
H'	0.56	0.84	0.94	0.69	0.59	0.39	0.52	0.78	0.42
D	0.62	0.49	0.42	0.46	0.68	0.76	0.72	0.54	0.74

Note: N: Individual abundance; S: Species abundance; R1: Abundance Index; H': Diversity index; D: Dominance Index

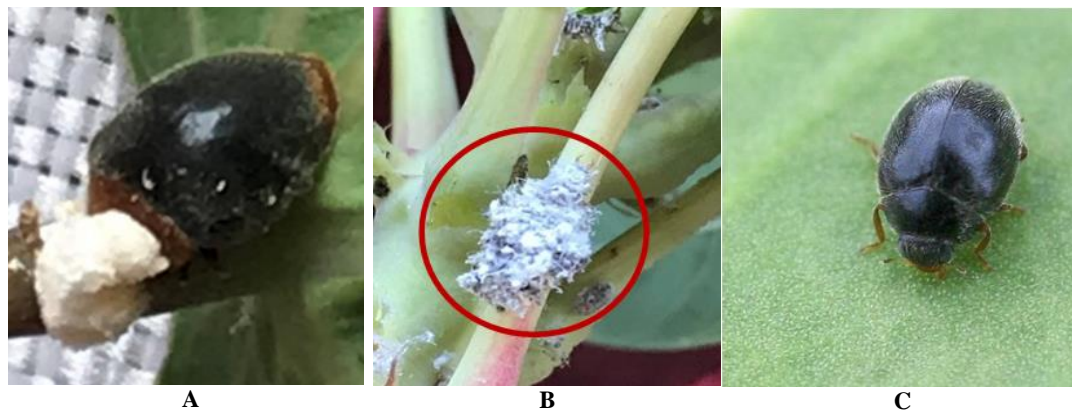


Figure 6. Predator fauna. A. *Cryptolaemus montrouzieri* adult (magnification: 5x); B. larvae instar-3 of *Chrysoperla carnea* (magnification: 5x); C. *Scymnus* sp. adult (magnification: 5x) (Personal documentation)

The investigation of the community structure of insect predators associated with the cassava mealybug *P. manihoti* in the field indicated that the values differed. Each predator species' population abundance was generally dominated by *C. montrouzieri* predator species, with 7-30 adults, followed by *C. carnea* predator species, with 1-10 adults, and *Scymnus* sp., with 2-6 adults. The abundance index values of predators were classified as low (3.5), with index values ranging from 0.54-0.76, while the diversity index values were classified as low (1.0), with values ranging from 0.39-0.94. Dominance index values range from moderate (>0.30-0.60) to high (>0.60-1.00), with values ranging from 0.42-0.76.

Cryptolaemus montrouzieri population dominance is assumed to be related to its adaptability and competitiveness with other predators. Furthermore, *C. montrouzieri* has a cannibalistic behavior toward each other. We suggest that the low abundance of predators *C. carnea* and *Scymnus* sp. is produced by biological variables such as the effect of hyperparasitoids that become natural enemies for predators *P. manihoti*, rather than abiotic factors such as height, temperature, and precipitation.

In summary, the morphological identification findings of the mealybug species attacking cassava in Bali were *Phenacoccus manihoti*, an exotic species that had never been documented in Bali previously. With a high population abundance, this pest has spread far and equally over all of Bali's regencies/cities. In all regencies/cities, the age groups of instar-1 and 2 nymphs dominated the population structure of *P. manihoti*, except in Buleleng Regency, where the age groups of instar-3 and 4 nymphs dominated. The Bangli Regency region had the greatest population density, while the Karangasem Regency area had the lowest. Polyphagous and cosmopolitan characteristics that easily adapt to the environment, as well as host plant factors (plant varieties, abundance of host plants in the field), farming methods (such as monoculture and intercropping), temperature, and rainfall factors, were the main factors influencing the *P. manihoti* population in the field. *Cryptolaemus montrouzieri*, *Chrysoperla carnea*, and *Scymnus* sp. were the insect predator fauna discovered in the field with the *P. manihoti* population. The predator

fauna community has a low index of diversity, abundance, and dominance structurally. *Cryptolaemus montrouzieri* was the most dominant of the three predator species in the field.

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