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The Role of *Plesiochrysa ramburi* (Shneider) (Neuroptera: Chrysopidae), to Control *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) at Cassava in West Java, Indonesia

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Abstract

Mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) is new invasive pest which have been attacked cassava in Indonesia. This pest can cause loss of yield about 80%. At this time in Indonesia cassava increasingly developed as a food and non-food industries, the pests will reduce the production of cassava if control measures are not taken. Biological control is one of alternative control which have developed using natural enemies such as parasitoid and predator. The result of cassava survey in field was showed that there are natural enemies were dominant is Chrysopidae *Plesiochrysa ramburi*. Potential experiment of *P. ramburi* in laboratorium was showed that eating preferences 3rd instar larvae of *P. ramburi* the second instar nymphs of *P. manihoti* has the highest preference index is equal to 0.159. Third instar larval stage is a phase most potential predators to control *P. manihoti* because it has the longest stage with an average length of instars 3-4 days. On treatment with a single-stage feeding prey (non-choice) showed that, three larval instars highest predation predator doing an average of 439.35 prey.

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While on treatment provision prey with diverse prey (choice) indicates that there is the composition of the selection of prey during the larval stage of *P. ramburi* on *P. manihoti* is 303.25 (The first instar), 285.45 (second instar), 102.2 (third instar), and 66.25 (adults). Third instar larvae functional response of *P. ramburi* on *P. manihoti* including the second type functional response.

Key words : *Plesiochysa ramburi*, *Phenacoccus manihoti*, cassava

1. Introduction

Biological control is the foundation of an integrated pest management (IPM). In principle, the IPM aims to minimize of synthetic chemicals that can cause negative impacts on the environment such as pest resistance, resurgence, the killing of non-target organisms, the emergence of secondary pests, the residue in plants and disruption of the surrounding environment such as water, soil and air [13].

P. manihoti can be controlled in several ways such as resistant plants, with organic chemicals, the cultivation and biological control. Mealybug control ever done is with a combination of host plant resistance and biological control [20]. The research in the United States and Africa, it is known that none of the cassava varieties that are resistant to *P. manihoti*, pest control is mainly done by conserving and augmentation of natural enemies such as has been done in Thailand [2, 3, 4] and [26], and in Africa [6]. In Indonesia the cassava plant, which is applied IPM components include (1) planting resistant varieties, (2) technical culture, (3) biological control, and (4) chemical control if needed [18].

One of the natural enemies that are found in cassava cultivation is of the order Neuroptera, Chrysopidae. Insects of the family Chrysopidae with common characteristics have greenish wings, eyes golden yellow or reddish with a length of approximately 12-20 mm, the eggs laid by the stalk on the substrate. Adult insects live freely, by eating honey dew and pollen. Larvae have a strong mandible, crescent-shaped function to prey on soft-bodied insects such as aphids, mealybug and others. Adults, active flying, especially in the afternoon and evening [27, 19].

Adults have a strong urge flight, and can fly for 3 to 4 hours each of their first two nights and laying on the fifth day after adult emergence. Egg-shaped oval, protective laid singly at the end / long silk ends, resembling miniature cattails that grow from the plant leaves, pale green, turning gray within 2-3 days. After 6-7 days the eggs hatch, the larvae are very active, have three instars, gray or brown, crocodile leather, with growing feet and growing mandibels like a big clamp to suck the body fluids prey. Larvae grow from <1 mm to 6-8 mm. After the third instar larvae rounded, then form pupae usually in hidden places in the plant. Adults emerge in 8-10 days [11]. The first instar approximately 3-4 days, 2nd instar 3-4 days, while the 3rd instar 5-6 days, and the pupa 14-15 days. The length of time of an adult, until the eggs laid back takes 40 days [8].

Effectiveness of *C. Carnea* in controlling aphid insects in some crops have been studied by several researchers, it is known that one larvae chrysopa able to prey on 500 aphids in their life [7, 10]. And its role as a predator in some pest controllers have also learned [23].

This study aims to determine the potential for predation of *Plesiochrysa ramburi* to mealybug *Phenacoccus manihoti* in the laboratory.

2. Materials and Methods

The study was conducted in the laboratory of Ecology Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, from July to October 2012.

Rearing of *Plesiochrysa ramburi*

Larvae of *P. ramburi* collected from cassava planting and maintained in a petri dish to become pupae. Pupae hatch into adults who will be transferred to the cage mesh size of 40x40x60 cm. Adults maintenance by providing a mixture of honey, yeast and water with a ratio of 1:1:1 [24] that serves as a substitute for nectar. Feed is applied to paper and taped to the wall confinement. Adults usually lay eggs on the surface of the cage or netting. After the eggs are laid soon separated in petri dish, until they hatch. Larvae feed on mealybug *P. manihoti*.

Rearing of *Phenacoccus manihoti*

P. manihoti larvae obtained from cassava plantation. Then the larvae infested cassava plants were grown in a plastic cup (height 16 cm, diameter 10 cm) in laboratory conditions.

1. Long larva stage *P. ramburi*:

from the rearing of predators taken 20 samples of *P. ramburi*, to determine the long period of 1st instar, 2nd instar, and 3rd instar of larvae.

2. Prey preferences of 3rd instar of larvae *P. ramburi*, at the stage of instar *P. Manihoti*.

At this treatment using 3rd instar of larvae *P. ramburi* and enter into a petri dish and then treated with the mealybug 1st instar to adults each 50 prey. Treatment conducted on 10 replicates. Observations were made by looking at the number of each instar *P. manihoti* remaining at 3, 6, 12, and 24 hours after the release of predators. Before treatment, predators were fasted for about 12 hours. Then calculated the degree of selection (preference index) for prey with the formula: $L_i = r_i - p_i$, L_i prey selection index, r_i = proportion of prey eaten by predators, and p_i = proportion of prey available. Maximum preference occurs when $r_i = 1$ and $p_i = 0$, and maximum rejection occurs if $r_i = 0$, and $p_i = 1$. If the value of L_i positive and approaching the maximum value of the preference, and if the value of L is negative, avoidance (rejection) to the maximum. Data analysis using SPSS Version 16.0. Then proceed with ANOVA, Duncan's multiple range test at the 5% level.

3. Predation potential of each level stadia larvae of *P. ramburi* on *P. manihoti*.

Treatment of non choice

Instar predators used were 1, 2 and 3. Petri dishes that had been supplied filled with chunks of fresh cassava leaves. After that into a petri dish infested 2nd instar of larvae *P. manihoti*, 450 nymphs prey for each treatment. Furthermore the different predator larvae instar put into a petri dish filled with prey. Observations were made every day until the predator to change to the next stadia. At each observation, the addition of cassava leaf if necessary. Treatment was repeated 20 replications. Counting the number of prey eaten conducted at the end of each stadia predator, by counting the number of remaining prey.

Treatment with choice

Instar predators used were 1, 2 and 3. Petri dishes are provided to put the cassava leaves with prey *P. manihoti*. For each treatment with different stadia instar larvae predators, prey included the four stadia instar nymphs with composition 1, 2, 3, and adults of amounted to 200 nymphs, so that the total number of prey is 800. After a petri dish filled with prey, predators then inserted in accordance with their respective stadia. Treatment was repeated 20 replications. A large number of prey eaten is calculated at the end of each period instar predators by counting the number of each prey remaining.

4. *P.ramburi* functional response of the mealybug *P. manihoti*:

To observe the predator functional response is used Completely Randomized Design (CRD) with 10 levels of prey density and 10 replications. Prey density is 1, 2, 4, 6, 8, 10, 20, 30, 40, 50. The second instar *P. manihoti* is put into a petri dish. Then the third instar larvae of *P.ramburi* who had fasted for 12 hours put into a petri dish which had contained prey at every level of density. Observations were made at intervals of 3 hours for 24 hours to the number of prey eaten. Prey additions to replace the lost or eaten prey according to its original density.

Analysis of the data used to determine the type of functional response of predators using the equation proposed by Juliano [22] as follows:

$$N_e = \frac{\exp(+ P_1 N_o + P_2 N_o^2 + P_3 N_o^3)}{1 + \exp (P_0 + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)}$$

Based on the above equation, N_o is the density of prey available, the proportion of prey consumed N_e , P_0 is the point of intersection, P_1 is the coefficient of linear, quadratic P_2 and P_3 is a cubic. The fourth parameter is alleged by the maximum likelihood method with the procedure PROC CATMOD SAS [21].

3. Results and Discussion

Long larva stage *P. ramburi*:

Active phase as predators of *P. ramburi* is larval, 1st to 3rd instar of larvae. One way to determine the effectiveness of a predator is to see the active period of eating and feeding capacity [15]. In the laboratory, a long period of larval *P. ramburi* is as follows (Figure 1).

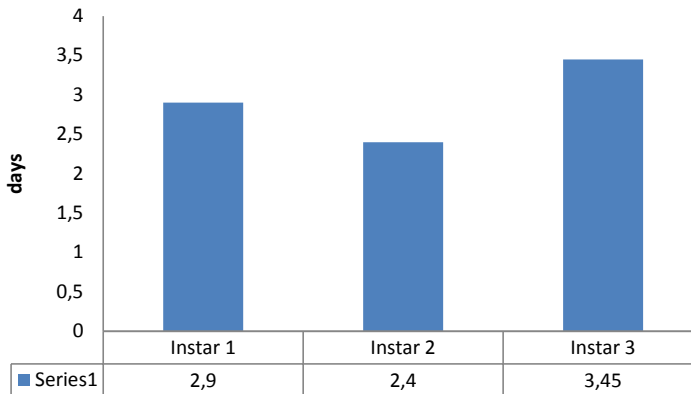


Figure 1. Long live larval stage is predator *P. ramburi*

The Fig. 1 shows that the active phase of predators eating lasted an average of 8-9 days, which is the longest phase in the third instar larval period (3-4 days). Larval molting experienced three times. 1st instar of larvae coming out of the egg is crawler with an average size of 1 mm (Figure 2). Then after 2-3 days, first instar molt and begin entering the 2nd instar of larvae with a body length of approximately 3-4 mm. Third instar larval body size reached 5-6 mm. Larva stage *P. ramburi* relatively short compared to Chrysopid in general as in the genus *Chrysoperla* sp. the immature stage at 21 days [8]. According to research [12], chrysopid kind *Apertochrysa* sp cultured on *Corcyra cephalonica* (Lepidoptera: Pyralidae), has a larval stage 17-18 days, a generation time of 40.6 days, females live longer up to 38 days, a long time from egg until the female dies achieve the 68 day. Development and reproduction of family Chrysopidae, potential to be developed in the laboratory, such as the one chysopid *Mallada boninensis*, cultured on *C. cephalonica*, fecundity has reached more than 139 individuals [25].

Feed preferences, 3rd instar of larvae *P. ramburi*

3rd instar of larvae are most active phase of the feed in the life period *P. ramburi*, the phase preference of predators on prey stadia are as follows (Table 1).

The preference index 3rd instar of larvae of *P. ramburi* is seen that the four stadia preferred prey by predators, evident from the preference index is positive. If the preference index close to 1, indicating that the stadia are increasingly favored prey by predators. Table 1 shows that the preference index of prey in the second instar (0.159) are highest compared to others, it can be said that the second instar stage of prey is most preferred by predators. Whereas prey on adults of phase has the lowest preference index (0.129). The high preference of

predators on second instar of pray, because this phase has a body size relatively small (length \pm 0.41 and width \pm 0.17 mm), with the structure of the outer shell is still soft making it easier for predators to catch and sucking host body fluids. Instead the low preference in adults because adult body size larger (length \pm 1.25 and width \pm 0.63 mm), and a tougher outer skin so that predators are more difficult to deal with using mandibel. Prey handling time difference can be seen in the figure below (Figure 3).

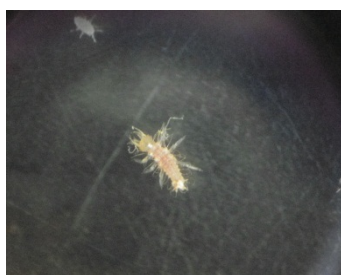
Table 1. Preference index 3rd instar of larvae of *P. ramburi* (D: Number of prey, Li: preferences Index)

Instar	Observation time (hours after the investment)							
	3		6		12		24	
	D	Li	D	Li	D	Li	D	Li
<i>P. manihoti</i>								
1 st instar	18	-0.090	24.4	-0.033	28.5	0.002	42 a	0.137
2 nd instar	10.3	-0.151	21.4	-0.045	30	0.037	42.7a	0.159
3 rd instar	5.6	-0.117	8.4	-0.050	11.7	0.029	16.3b	0.138
Adults	2.7	-0.132	4.5	-0.053	7	0.056	9.2c	0.129

Eggs



1st instar of larvae



2nd instar of larvae



3rd instar of larvae



Pupa



Adults



Figure 2. Phase of life *P. ramburi*

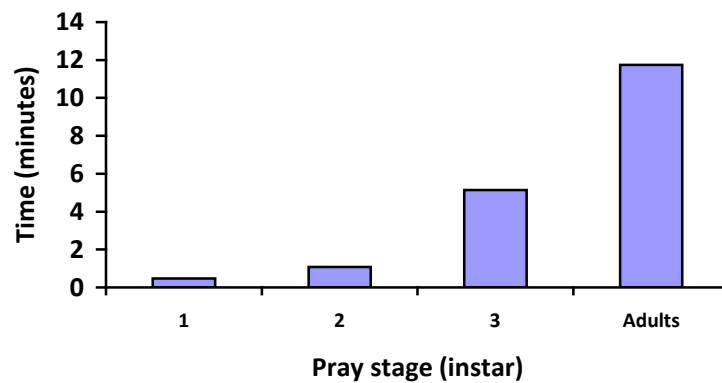


Figure 3. Handling time 3rd instar of *P. ramburi* on stadia of *P. manihoti* during the 2 hours after predator fasted.

The length of time handling prey is seen that the time taken to deal with the first and second instar prey is not different from that (0.476 and 1.079 minutes), but it appears that predators prefer prey on second instar compared with the first instar (Figure 4). The first instar crawlers are one of the factors that cause the predator prefers 2nd instar, although for the first instar prey needed to tackle a fast enough time.

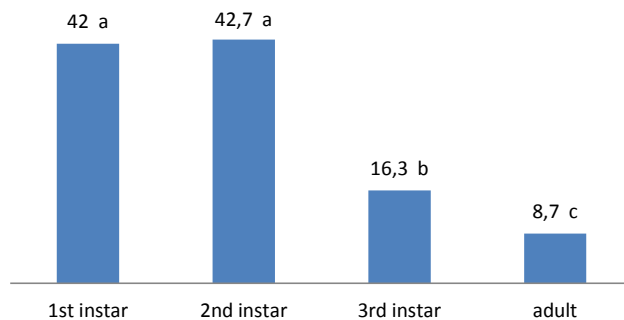


Figure 4. The average number of each instar prey on feed after 24 hours by 3rd instar of *P. ramburi*.

In the experiments conducted [17] argued that *Chrysoperla Carnea* experienced many deaths when fed with third instars of *Aphis*, as well as the first instar and third instar of *Myzus persicae*. It is related to the nutrition found in the prey of predators that affect survival.

Potential predation *P. ramburi*

Treatment of non choise

Potential predation from *P. ramburi* at each larval stage were given a second instar *P. manihoti* can be seen in the figure 5.

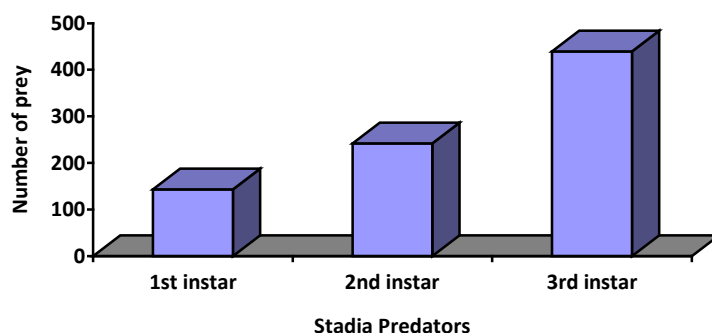


Figure 5. The mean number of second instar of *P. manihoti* were wiped out by larval stage of *P. ramburi*.

Number of prey eaten by the third instar predator is much higher than the previous instar. The average number of predation third instar of larvae reached 439.35 during the period 3rd instar of larvae (3-4 days). The high number of prey eaten due to high nutrient needs during this period before entering the pupal period. A relative large body size (approximately 5-6 mm in length) require more food than younger instar period. First instar and second instar predators capable of consuming an average of 143.35 and 242.1 second instar nymphs of *P. manihoti*, during the period that is approximately 2-3 days. The average total number of predation from first instar to third instar *P. ramburi* could reach 824.80 second instar nymphs of *P. manihoti*. Potential predation *P. ramburi* relatively higher compared to other predatory species Crysopid as *Chrysoperla sp* were only able to prey on aphids 100-600 [7, 10, 14].

Treatment with choice

Provision of prey with different stadia indicates that the composition in the amount of prey feed by *P. Ramburi* vary at each stadia predators. But generally seen that the first instar and second instar prey *P. manihoti*, be selected more predation by *P. ramburi*. Tendency of prey selection by first instar of *P. ramburi* different tendencies prey selection by the second and third instars of the predator (Figure 6). The first instar *P. ramburi* more prey on 1st instar of larvae *P. manihoti*, because it has a smaller body size (approximately 1-2 mm). Selection of prey is also associated with the ability to handle a prey predator. Potential predation *P. ramburi* all instars stages of *P. manihoti* reached approximately 757, 15 prey.

Functional response predator *P. ramburi* against mealybug *P. manihoti*:

Functional response in general can be used as a measure of the effectiveness of a predator. Functional response can be divided into several types. According to [5], the functional response can be divided into 4 types. But the division of the functional response types into 3 types as proposed by [1], more frequently used. Based on the equation [22], by calculating the proportion of prey consumed compared to the available prey obtained linear coefficients (P1) <0 is -0.00042 for functional response in *P. ramburi*, where the proportion of prey consumed decreased with increasing prey density (Figure 8).

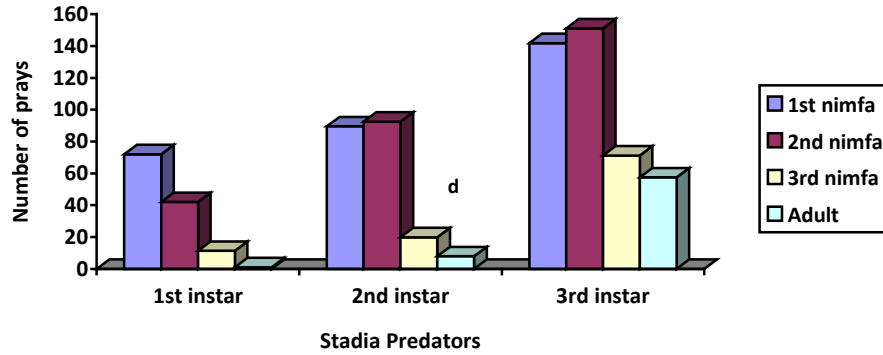


Figure 6. Ability predation by larval stage of predator *P. ramburi*

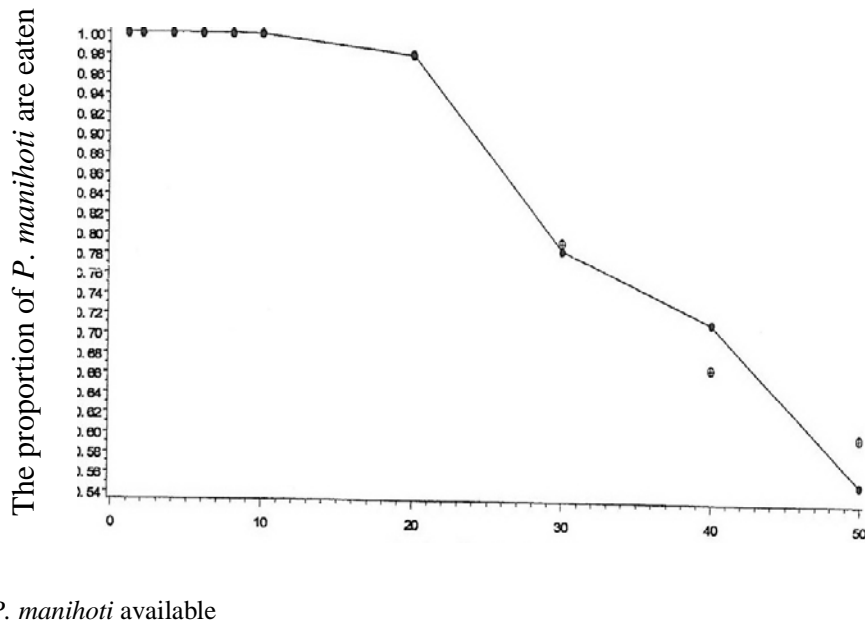
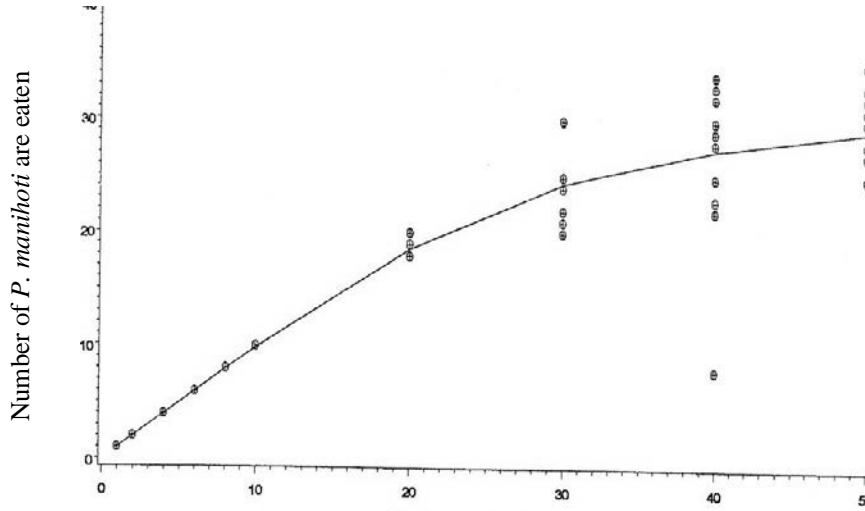


Figure 7. The average proportion of prey eaten at several densities prey

By using the maximum-likelihood analysis, functional response types obtained from *P. ramburi*, which is a function of the number of *P. manihoti* are preyed upon by many *P. manihoti* available. This shows that the type of functional response is type II (Figure 9). According to [1], there are three types of functional response, type I is a linear functional response type where predation rate increased or decreased in relation to the increase or decrease in prey populations. Type II functional response, often called the hyperbolic functional response in which the rate of predation decreases with increasing prey density. Type III, also known as the sigmoid functional response type, where the predation rate was initially slow, then increased at a rate more quickly,

before it finally became constant. The first type is usually found in passive predators just as the spider. While type II, commonly occurs in laboratory experiments, with particular prey [9].



Number of *P. manihoti* available

Figure 8. Type functional response curve of *P. ramburi*

In general it can be said that the predator *P. ramburi* enough effective because of the nature of the type II functional response criteria or hyperbolic functional response is when the rate of predation decreases with increasing prey density, prey maximum mortality occurred at low prey densities. This is consistent with one of the criteria for determining the effectiveness of a predator that can be measured by its ability to find prey at low prey densities and consume many prey upon abundant prey populations [16].

4. Conclusion

The active phase of the predator eating average of 8-9 days, which ate the longest phase occurred during 3rd instar (3-4) days, with the highest preference index (0.159) in the second instar prey stadia. On treatment with one stadia feeding prey (non-choice), suggests that predation is highest in the third instar larvae of *P. ramburi* with an average of 439.35 prey. Treatment provision prey with diverse prey (choice) indicates that there is the composition of prey during the larval stage on *P. ramburi* to *P. manihoti* is 303.25 (1st instar), 285.45 (2nd instar), 102.2 (3rd instar), and 66.25 (adults). Functional response of *P. Ramburi* on *P. Manihoti* including the type 2 functional response.

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