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Suitability of non-natural prey during the larval growth of *Heteroneda billardieri* (Crotch), a potential predator of mango leafhopper

[Kesesuaian mangsa bukan semulajadi sepanjang tumbesaran larva *Heteroneda billardieri* (Crotch), pemangsa berpotensi benah mangga]

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Abstract

Mango leafhopper (MLH), *Idioscopus clypealis* Lethierry (Hemiptera: Cicadellidae) is naturally controlled by a predator beetle named *Heteroneda billardieri* (Crotch) (Coleoptera: Coccinellidae). Mango leafhopper is used to rear *H. billardieri* with regards to biological control efforts. However, an alternative prey is needed in order to boost the mass rearing process of the beetle. Therefore, different prey species were selected along with artificial diets to study their effect on the growth and development of *H. billardieri* larvae. Two non-natural prey species, the cowpea aphid, *Aphis craccivora* Koch and leucaena psyllid, *Heteropsylla cubana* Crawford were tested along with MLH and artificial diets. All types of diets were accepted by the predator but their suitability was varied. The artificial diets were not suitable for larval development and psyllid was the least suitable when provided alone. However, simultaneous feeding of prey together with artificial diets significantly affected growth and development of *H. billardieri* compared to feeding with a single prey or artificial diets only except when the larvae were fed with aphids alone. The larval development and survival were best when fed just with a single diet of aphids suggesting that aphids can serve as the best alternative non-natural prey during the larval stage of growth, especially when the availability of MLH is limited.

Key words: biological control, diet, predatory lady beetle, prey suitability, larval developmental rate, larval survivorship, mass rearing

Introduction

For insects especially in the larval stage, choice of diet is crucial to ensure their survival into adulthood. Entomophagous predators that are often used for biological control of insect pests are commonly required to be cultured and mass reared in the laboratory. In these species, food items must also be mass reared in the laboratory. However, there are instances when rearing the host or prey is problematic and alternatives that are easier to rear are used.

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In Laguna, Philippines, a lemon-yellow netted lady beetle identified as *Heteroneda billardieri* (Crotch) was observed to be a potential predator for mango leafhopper (MLH), *Idioscopus clypealis* Lethierry (Hemiptera: Cicadellidae) (Adorada 2006). Mango leafhopper is not only an important pest of mango in the Philippines but also in other countries in the Southeast Asian region. It is the most common and destructive species of hoppers which cause heavy damage to mango crops (Varshneya and Rana 2008).

Heteroneda billardieri are ladybird beetles (Coccinellidae: Coleoptera) that are predaceous for both larvae and adults. Adult beetles of *H. billardieri* have an oval convex body. Elytra are yellow-lemon in colour with black markings resembling a network pattern (*Plate 1*). The larvae have an elongated and flattened body with six fully developed legs (*Plate 2*). They pass through four larval instar stages. Being a predatory of coccinellids, they may vary widely in their spectrum of diets (Hodek and Honek 1996; Harwood and Obrycki 2005; Hodek and Honek 2009).

In mass production of *H. billardieri* as a biological control agent, one of the limiting factors is the difficulty experienced in rearing sufficient quantities of prey. Foremost of which is the seasonality of MLH itself. Mango leafhopper can be considered as a seasonal pest because the females usually lay more eggs and nymphs develop faster only during the flowering period of the mango. On non-flowering mango trees, MLH populations are quite low and rather difficult to detect (Smith 2008). This will limit the food supply for *H. billardieri* in the laboratory especially during off season in the field.

To address this problem, non-natural prey type or prey substitution in the rearing laboratory is considered. For this study, two non-natural preys were used to test their suitability and their effect on the predatory beetle growth and development together with its natural prey, MLH, and also the artificial diet in terms of their mass rearing possibilities. Cowpea aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae) and leucaena psyllid, *Heteropsylla cubana* Crawford (Hemiptera: Psyllidae) were used as prey substitutes for rearing of *H. billardieri* because of their availability in the field all year round. Having diverse preys, whether natural or unnatural, is better for a predator's growth, development and survival. In particular, this study was conducted to test the hypothesis that larval growth on diets of two non-natural preys (aphids and psyllids) was as active in terms of growth compared to when they consume natural prey (MLH). The study was also aimed to test the hypothesis that mixed diets given simultaneously would enhance larval growth better than single diets alone.

Plate 1. Adult beetles of Heteroneda billardieri

Plate 2. Larva of Heteroneda billardieri (3rd larval instar)

Materials and methods

Location and duration of study

The study was conducted at the Insect Ecology Laboratory, Crop Protection Cluster, University of the Philippines Los Baños (UPLB), College, Laguna (14° 9' 54.18" N, 121° 14' 29.55" E) from January until the end of May 2011.

Collection and rearing of study insects

Heteroneda billardieri adults were collected from mango trees (*Mangifera indica*) along the Institute of Plant Breeding Road, Los Baños, Laguna and UPLB campus. The adults were brought back to the laboratory and kept in rearing cages (50 cm x 50 cm x 50 cm).

The field collected *H. billardieri* adults were maintained at 23 ± 1°C with a photoperiod of 10:14 (L:D). They were fed with an overabundance or *ad libitum* (feeding management through which the animals are offered as much food as they want) of either leucaena psyllids (*H. cubana*), mango leafhoppers (*I. clypealis*) or cowpea aphids (*A. craccivora*). Leucaena psyllids were collected from ipil-ipil trees (*Leucaena leucocephala*), mango leafhoppers were collected from mango trees (*M. indica*) and cowpea aphids were collected from cowpea beans (*Vigna unguiculata*). Besides insect prey, an artificial diet containing a mixture of infant formula and brown sugar (4:1) were also provided to the adults of *H. billardieri*. Water was provided in a small cup with a cloth stopper as their drinking source.

In order for the female adults to lay eggs, cotton was placed inside the corner of the rearing cage as a substrate. All laid eggs were counted and transferred to new petri dishes to avoid any egg cannibalism by the adult beetles. The eggs were monitored daily for hatching. Upon hatching, first instar (F1) larvae from the same cohort were placed individually in a 250-ml container to elude cannibalism and were used for the experiment.

Artificial diet

Infant formula (Brand S-26 Gold) manufactured by Wyeth Philippines Inc. was used as one of the diets given in this study. The mixing of infant formula and brown sugar was done in a ratio of 4:1. The diet mixture was kept in a small container for use later in the experiment.

Experimental set up

Twenty newly hatched *H. billardieri* larvae were assigned randomly into 7 different 250-ml containers to receive one of the following treatments as experimental diets which were provided throughout the larval stages of *H. Billardieri*.

1. Treatment A - MLH only
2. Treatment B - Aphids only
3. Treatment C - Psyllids only
4. Treatment D - Artificial diet only
5. Treatment E - MLH and artificial diet
6. Treatment F - Aphids and artificial diet
7. Treatment G - Psyllids and artificial diet

The combination diet of prey species and artificial diet were given at the same time for Treatments E, F and G. The larvae were examined daily for moulting. Dead prey along with exuviate of the larvae were removed. Fresh experimental diets were provided every day. The duration for each immature stage from first larval instar until adult emergence and pupal weight were recorded. Number of dead larvae, number of larvae which successfully pupated and number of larvae which emerged as adults were also recorded.

Statistical analysis

Completely randomised design (CRD) was used in this experiments with 20 replications. The Statistical Analysis System version 9.1.3 (SAS Institute 2003) was used to analyse the data. The influence of different experimental diets on each immature stage duration, total larval duration, total pupal duration, total immature duration and pupal weight were examined by using Paired T-test (PROC TTEST in SAS). Analysis of variance (ANOVA) (PROC GLM in SAS) was used to analyse larval survival and percentage of pupated larvae. In calculating percentages, data were transformed to Arc Sine prior to analysis. Significant means were compared and separated using LSD Test (α = 0.05). Life test analyses were carried out using PROC LIFETEST in SAS to compare total immature duration among treatments. Wilcoxon test was used to determine equality among treatments.

Survival probabilities were computed using Regression with life procedure in Minitab version 16 (Minitab 16 Statistical Software 2010).

Results

Rate of development

Duration of each larval and pupal stage of *H. billardieri* fed with different types of experimental diets were compared (*Figure 1*). Survival for every treatment in each immature stage varied and for comparing the significance in difference among treatments, Treatment D was set as the control because survival rates in every immature stage were the lowest (*Table 1*).

The duration of first instar for all treatments had no significant difference although first instars fed with the artificial diet only (Treatment D) took the most days to moult into the next instar stage. Few individuals (5 out of 20) survived to the second instar on the artificial diet alone.

For the second instar, larval development was the shortest for Treatment B (aphid only) where a total of 19 individuals moulted into third instars. The mixed diets (Treatments E, F and G) and Treatment A (MLH only) resulted in a shorter duration compared with the diet of psyllids or artificial diet only. However, there were no significant differences among all treatments.

Third instar larvae fed with all simultaneously given mixed diets (Treatments E, F, and G) and diet of aphids

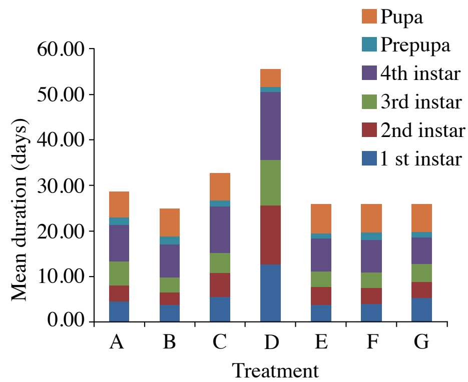

Figure 1. Development period of immature stages of H. billardieri fed with different mixed diets given simultaneously. Comparisons of each immature stage among treatments were done using T-Test (α = 5). A = MLH only, B = Aphids only, C = Psyllids only, D = Artificial diet only, E = MLH + Artificial diet, F = Aphids + Artificial diet, G = Psyllids + Artificial diet

alone (Treatment B) showed no significant difference but had shorter duration compared with the rest of the treatments. However, there was no significant difference between larvae fed with diets in Treatments G (psyllids and artificial diet) and C (psyllids only). In Treatment D, only one individual survived to this stage and its duration was significantly longer compared with the other treatments.

As for larval duration of the fourth instar, there was no significant difference between single diets of either MLH or aphids, but both were significantly shorter compared with other single diet treatments. Feeding on these two preys however, were not significantly different from feeding with the mixed diet treatments except for Treatment G. Psyllids when provided together with the artificial diet had a better effect on the fourth instar duration than when provided alone.

Larvae fed with the artificial diet alone had the fastest rate of pupation when compared to the different diet regimes. Treatment D had significantly lower number of development days as a pupa before adult hatching.

Table 1. Duration of immature stages of *H. billardieri* fed with different mixed diets given simultaneously (Comparison within treatments)

Treatment (N = 20)	Duration (days) Mean ± (SE)											
	n	1st instar	n	2nd instar	n	3rd instar	n	4th instar	n	Prepupa	n	Pupa
A - MLH only	19	4.42 ± 0.27^{a}	17	3.65 ± 0.23^{a}	16	5.25 ± 0.39^{b}	14	7.93 ± 0.38^{b}	12	1.75 ± 0.13^{a}	12	5.67 ± 0.19^{b}
B - Aphids only	19	3.63 ± 0.16^{a}	19	2.89 ± 0.13^{a}	18	3.33 ± 0.18^{d}	18	7.17 ± 0.39^{b}	17	1.82 ± 0.10^{a}	14	6.14 ± 0.14^{ab}
C - Psyllids only	18	5.5 ± 0.39^{a}	12	5.25 ± 0.46^{a}	10	4.5 ± 0.27^{bc}	7	10.14 ± 0.99^{a}	5	1.4 ± 0.24^{a}	4	6 ± 0.00^{b}
D - Artificial diet only	5	12.6 ± 3.47^{a}	2	13 ± 4.00^{a}	1	10^{a}	1	15^{a}	1	1^{c}	1	4^{c}
E - MLH + artificial diet	16	3.75 ± 0.14^{a}	16	3.94 ± 0.49^{a}	15	3.53 ± 0.24^{d}	15	7.07 ± 0.32^{b}	14	1.21 ± 0.11^{c}	13	6.46 ± 0.18^{a}
F - Aphids + artificial diet	17	4 ± 0.26^{a}	16	3.44 ± 0.13^{a}	15	3.53 ± 0.19^{d}	15	7.07 ± 0.36^{bc}	15	1.6 ± 0.13^{a}	14	6.36 ± 0.13^{a}
G - Psyllids + artificial diet	16	5.38 ± 0.18^{a}	15	3.47 ± 0.19^{a}	15	3.87 ± 0.20^{cd}	14	5.86 ± 0.21^{c}	14	1.29 ± 0.13^{bc}	14	6.14 ± 0.10^{ab}

Means with same letters in the same column are not significantly different at T-Test (α = 0.05). Equality of variances tests were done to determine which method to use for calculation of t value. (Pooled method for equal variances or Satterthwaite's method for unequal variances)

H. billardieri larvae fed with aphids only (Treatment B) recorded the shortest total larval duration followed by Treatments F, E, G, A, C, and D (*Table 2*). Total larval duration for all treatments with mixed diets was significantly shorter than treatments with single diets alone (Treatments A and C) except for Treatment B, which was not significantly different from larvae fed with simultaneously given mixed diets of aphids plus artificial diet and MLH plus artificial diet (T = 1.78; P = 0.0871, T = –1.31; P = 0.2115). Larvae fed with psyllids plus artificial diet (Treatment G), however, was significantly different from Treatment B (T = – 4.04; P = 0.0004). There were no significant differences among the mixed diets. Larvae fed with artificial diet alone had the longest larval duration, significantly different from the rest of the treatments.

Treatment D recorded the shortest total pupal duration and differed significantly from the rest of the diet regimes (*Table 2*). For each and every prey species, there was no significant difference in total pupal duration when given the diet regimes either solely, or mixed with artificial diet.

In terms of overall development time of immatures, Treatment B was the shortest but not significantly compared to Treatments E and F (T = – 0.98; P = 0.3459, T = – 1.65; P = 0.1106). There was no significant difference between the three mixed diets (Treatments E, F, and G) but they were significantly different from the three single diets (Treatments A, C and D) (*Table 2*). *Figure 2* shows the emergence rate of the immatures. When fed with the diet of Treatment B, 100% of the surviving immatures became adults in less than 25 days. For Treatment D, which resulted in the lowest survival rate, the larvae took the longest time to emerge as adults (60 days). All the treatments were significantly different from one another by Wilcoxon test (X^2 = 30.08; P <0.0001).

Table 2. Larval and pupal development periods of *H. billardieri* as affected by different mixed diets given simultaneously at the larval stage

Treatment (N = 20)	N	Duration (days) Mean ± (SE)		
		Larval	Pupal	Immature
A - MLH only	12	21.67 ± 0.20[c]	7.42 ± 0.15[b]	29.08 ± 0.68[c]
B - Aphids only	14	16.79 ± 0.33[e]	8.00 ± 0.10[a]	24.79 ± 0.32[e]
C - Psyllids only	4	25.75 ± 0.63[b]	7.25 ± 0.25[b]	33.00 ± 0.41[b]
D - Artificial diet only*	1	55·00[a]	5·00[c]	60·00[a]
E - MLH + artificial diet	13	18.38 ± 1.17[de]	7.69 ± 0.17[ab]	26.08 ± 1.28[de]
F - Aphids + artificial diet	14	17.86 ± 0.50[de]	7.93 ± 0.13[a]	25.79 ± 0.52[de]
G - Psyllids + artificial diet	14	18.57 ± 0.29[d]	7.43 ± 0.14[b]	26.00 ± 0.38[d]

Means with same letters in the same column are not significantly different at T-tests ($\alpha = 0.05$).
*Only one individual survived to complete the larval stage, pupate, and develop into an adult.
Equality of variances tests were done to determine which method to use for calculation of t value.
(Pooled method for equal variances or Satterthwaite method for unequal variances).
Means included only individuals surviving to adulthood

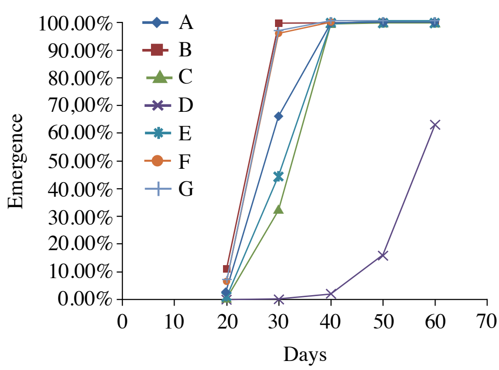

Figure 2. Rate of adult emergence for immature H. billardieri fed with different mixed diets given simultaneously. A = MLH only, B = Aphids only, C = Psyllids only, D = Artificial diet only, E = MLH + Artificial diet, F = Aphids + Artificial diet, G = Psyllids + Artificial diet. (Graph was derived from the survival probabilities Regression with life procedure of Minitab)

Survival to adulthood and to pupal stage

The rate of individual survival between single diets and simultaneously given mixed diets were compared as shown in *Figure 3*. High mortality was recorded at the first instar stage when the larvae were placed on artificial diet only (Treatment D). Only one larva survived to develop into a pupa and emerge as an adult. Nineteen could not even reach the third instar stage. As for Treatment C, which involved feeding with psyllids alone, only four out of 20 larvae emerged as adults. The survival rate went down drastically when the larvae entered the second instar stage. Survival rates in the rest of the treatments were quite similar especially among the mixed diets. For Treatment B (aphids only), 90% of the total individuals survived.

Figure 4 shows that 70% of *H. billardieri* larvae completed their development when provided with only aphids diet, aphids with artificial diet and psyllids with artificial diet with no significant difference among the treatments. These three treatments were also not significantly different from the larvae fed with MLH only and MLH with artificial diet (60% and 65% of survivorship respectively) and between 12 and 14 of them became adults (*Table 2*). Larvae fed with only psyllids diet (Treatment C) had low survival rate but was slightly higher than those undergoing Treatment D. The survival percentage for Treatment D was the lowest.

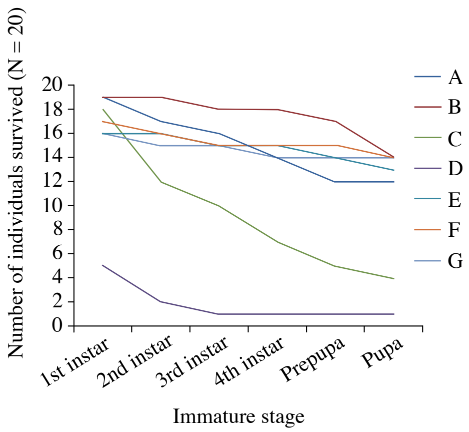

Figure 3. Individual survival rate of H. billardieri fed with different mixed diets given simultaneously. A = MLH only, B = Aphids only, C = Psyllids only, D = Artificial diet only, E = MLH + Artificial diet, F = Aphids + Artificial diet, G = Psyllids + Artificial diet

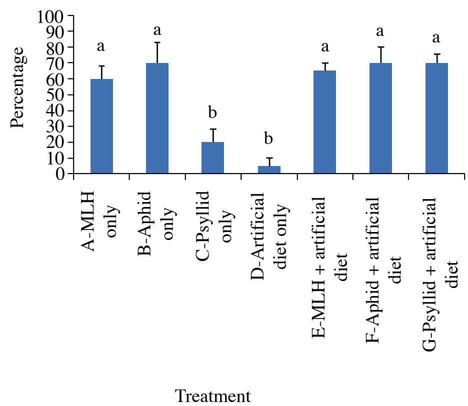

Figure 4. Survival of H. billardieri larvae fed with different mixed diets given simultaneously. Means with same letters indicate no significant difference at LSD test (α = 0.05). Data were transformed to Arc Sine prior to analysis

The difference for both Treatments C and D was highly significant from Treatments B, F, and G (*F* = 9.73; *P* <0.0001).

The percentage of larvae that successfully pupated is shown in *Figure 5*. Eighty-five percent larvae pupated when fed with aphids alone (Treatment B) but showed no significant difference compared to Treatments E, G, F and A (70, 70, 65, and 60% respectively). All Treatments A,

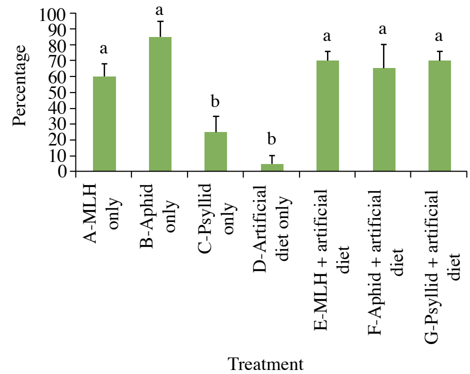

Figure 5. Percentage of pupation by H. billardieri larvae fed with different mixed diets given simultaneously. Means with same letters indicate no significant difference at LSD test (α = 0.05). Data were transformed to Arc Sine prior to analysis

B, E, G and F differed significantly from Treatments D and C (*F* = 8.60; *P* <0.0001). Treatment D recorded the lowest percentage (5%) followed by Treatment C (25%). There was no significant difference between Treatments D and C.

Pupal weight

Pupa from larvae fed on diet of Treatment G recorded the heaviest average pupal weight but showed no significant difference from Treatments F and B (*T* = – 0.87; *P* = 0.3923, *T* = – 1.30; *P* = 0.2052) (*Table 3*). Among the mixed diets, Treatment F and G had moderately significant differences compared with Treatment E (*T* = – 2.92; *P* = 0.0073, *T* = – 3.57; *P* = 0.0015). Pupal weight was not significantly different when the larvae were fed either with MLH alone (Treatment A) or MLH mixed with artificial diet (Treatment E) (*T* = – 0.59; *P* = 0.5608). The mixed diets in Treatments F and G recorded heavier average pupal weight compared with that of the single diets (Treatments A, C and D) with the exception of the single aphids diet (Treatment B). Treatment D had the lowest average pupal weight but showed no significant difference from Treatment C (*T* = 1.23; *P* = 0.3070).

Table 3. Pupal weight of *H. billardieri* fed during the larval stage with different mixed diets given simultaneously

Treatment (N = 20)	n	Pupal Weight (mg) Mean ± (SE)
A - MLH only	12	17.50 ± 0.65[b]
B - Aphids only	14	20.07 ± 0.65[a]
C - Psyllids only	4	14.25 ± 1.55[c]
D - Artificial diet only	1	10·00[c]
E - MLH + artificial diet	13	18.00 ± 0.55[b]
F - Aphids + artificial diet	14	20.50 ± 0.64[a]
G - Psyllids + artificial diet	14	21.36 ± 0.75[a]

Means with same letters are not significantly different at T-tests ($\alpha = 0.05$). Equality of variances tests were done to determine which method to use for calculation of t value. (Pooled method for equal variances or Satterthwaite method for unequal variances). Means included only pupae that survived to adulthood.

Discussion

Prey suitability is an important factor in prey selection especially for mass rearing in laboratories. As mentioned by Hodek (1973), the criteria used to determine prey suitability of a single prey species is the completion of preimaginal development and successful egg production. For a prey species or non-prey food to be suitable, it must provide all nutrients in balanced proportions and concentrations to meet a predator's metabolic requirements (House 1966).

Up to date, only limited studies have been done on the biology and ecology of *H. Billardieri*. A study done by Adorada (2006) was mainly focused on its taxonomy and classification. Recently, few studies have been carried out especially regarding the effect of different prey or non-prey items on the *H. billardieri* larval development (Medina and Velasco 2008; Barcos et al. 2014; Badrulhadza et al. 2018). These studies were undertaken for the purpose of developing a mass rearing protocol for the predator and specifically to determine the suitability of natural and non-natural diets or prey to the beetle for its larval survival and development.

Based on the results, simultaneous feeding of prey plus artificial diet significantly affected growth and development of *H. Billardieri* compared with feeding on single prey or artificial diet only. According to Badrulhadza et al. (2018), each food regime influenced the development time of each immature stage, total immature duration, survival to adulthood, percent pupation and pupal weight. In this study, it was found that the immature development into adults was prolonged up to 60 days with only 5% survivability when fed only with the artificial diet. Average pupal weight was also the lowest. This showed that the artificial diet was unsuitable for larval development and can cause high mortality. Diet on psyllids alone was least preferred when compared with MLH and aphids diets. A combination of three types of prey species with artificial diet given simultaneously, shortened total immature duration, increased survivability and percentage of larvae that developed into pupa and produced heavier pupal weight. These mixed diets were better than single diets of MLH and psyllids except when the larvae were fed with aphids alone. The immature duration was the shortest among all diet regimes when fed with aphids alone and adequate to support larval development without the need to mix with the artificial diet. This finding was similar to a study done by Badrulhadza et al. (2018) where *H. billardieri* performed well in terms of survival and reproduction if fed with different prey species rather than a single diet alone.

This study also indicated that psyllids constituted only an alternative prey in the physiological sense for *H. billardieri* larvae. *Heteroneda billardieri* larvae cannot develop solely on a diet of psyllids because they may not have the required nutritional value. Furthermore, the predaceous larvae may not be used to consuming psyllids because in nature, psyllids are not found on mango trees. According to Barcos et al. (2014), the search for prey by its predator was influenced by the presence of the prey on the plants. Barcos et al. (2014) also stated that *H. billardieri* oriented themselves to mango trees that were infested by MLH and not psyllids. Psyllids may also be less preferred due to its size and mobility. As mentioned by Sabelis (1992), most generalist predators select their prey according to their relative size. Psyllids could have been overlooked by the larvae due to their small size and escaping abilities.

This study also revealed that *H. billardieri* larvae survival increased and had faster developmental rate when fed with MLH as well as aphids even though aphids are not its natural prey. The lower mobility of aphids observed during the experiment could also lead to better capture success by the *H. billardieri* larvae. It can be considered that MLH and aphids are essential and suitable for the larval stage of this species.

Using aphids as an alternative source of diet is the best choice in mass rearing of *H. billardieri* at larval stage especially when the main natural prey is scarce or not available in the field. Moreover, MLH is not easy to rear because of the seasonality characteristic of the host plant. Aphids are easier to rear on legumes, either in the field or in the glasshouse. Aphid's parthenogenetic reproduction can provide a high number of individuals in a shorter period of time. This will adequately support the *H. billardieri* population in the insectary. Hopefully, by using aphids as an alternative prey species alongside MLH, high laboratory populations of the predator can be attained and maintained for future mass rearing studies or mass release in mango fields to control the target pest.

Conclusion

Being a generalist, *H. billardieri* readily accepts the two non-natural preys, leucaena psyllid (*H. cubana*) and cowpea aphid (*A. craccivora*), and the artificial diet (mixture of infant formula and brown sugar) along with its natural prey, MLH. However, their suitability for growth and survival varied. The artificial diet was not suitable for larval development and psyllids were the least suitable when provided alone. However, when given simultaneously a mixture of artificial diet with each prey species, the combined diet became more suitable and survival of the larva increased. Larval development and survival were best when fed with aphids alone. This showed that larval growth on a diet of non-natural prey (aphids) was as active as growth on natural prey (MLH). It can therefore be recommended that aphids can be used as an alternative source of prey alongside MLH in mass rearing of this predatory beetle.

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Abstrak

Benah mangga, *Idioscopus clypealis* Lethierry (Hemiptera: Cicadellidae) dikawal secara semulajadi oleh kumbang pemangsa *Heteroneda billardieri* (Crotch) (Coleoptera: Coccinellidae). Benah mangga digunakan untuk membiak *H. billardieri* bagi tujuan kawalan biologi. Walau bagaimanapun, mangsa alternatif diperlukan untuk menggalakkan proses penternakan besar-besaran kumbang tersebut. Oleh sebab itu, beberapa spesies-spesies mangsa berbeza telah terpilih beserta diet tiruan untuk menguji kesan ke atas tumbesaran dan perkembangan larva *H. billardieri*. Dua spesies mangsa bukan semulajadi, afid kekacang (*Aphis craccivora* Koch) dan kutu lelompat petai belalang (*Heteropsylla cubana* Crawford) telah diuji bersama-sama dengan benah mangga serta diet tiruan. Kesemua jenis diet dimakan oleh pemangsa tetapi kesesuaiannya berbeza-beza. Diet tiruan tidak sesuai manakala kutu lelompat pula adalah kurang sesuai untuk perkembangan larva sekiranya diberikan secara tunggal. Walau bagaimanapun, mangsa yang diberikan secara serentak bersama diet tiruan dapat mempengaruhi tumbesaran dan perkembangan *H. billardieri* secara signifikan berbanding mangsa atau diet tiruan yang diberikan secara tunggal semata-mata. Hal ini berkecuali sewaktu larva diberikan afid secara tunggal. Perkembangan dan kemandirian larva berada pada tahap terbaik semasa diberikan diet tunggal afid dan ini membolehkan afid digunakan sebagai mangsa bukan semulajadi alternatif untuk peringkat larva sewaktu ketiadaan benah mangga.