An Improved Artificial Diet for Rearing *Propylea japonica* (Coleoptera: Coccinellidae) Larvae[1]

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Abstract *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) is an important natural enemy of insect pests of agricultural importance. An artificial diet is needed when insects upon which the predators feed are in limited supply. Larval mortality, duration of larval development, duration of the pupal stage, and adult weight were compared for various concentrations of nine ingredients of an artificial diet in an $L_9(3)^4$ multi-index orthogonal array design. A range analysis ("R" method) coupled with an integrated balance method determined that the theoretically optimal diet for *P. japonica* larvae was 6 g ground powder of *Mythimna separata* (Walker), 2 g yeast extract, 1 g sucrose, 0.08 g olive oil, and 29.42 g basic diet. After further testing, we found that this diet yielded a higher larval survival rate, longer larval period, longer pupal period, greater adult weight, and higher eclosion rate of *P. japonica* compared with the eight artificial diets tested.

Key Words *Propylea japonica*, artificial diet

Propylea japonica (Thunberg) (Coleoptera: Coccinellidae) is globally distributed and is an effective natural enemy of various pests (e.g., aphids, planthoppers, and leafhoppers) and has potential for development as a biocontrol agent (Guadalupe et al. 2016, Zhang et al. 2012). Developing a suitable artificial diet to guarantee the rearing success of *P. japonica* is a key factor in realizing this potential. Previous studies have screened and identified nine dietary ingredients (i.e., honey, vitamin B, vitamin C, methylparaben, potassium sorbate, aphid powder, agar, Wesson's salt, and water) of an artificial diet that might be used for a sustainable food supply for *P. japonica* during times when aphids and other suitable prey are not available or are in limited supply (Chen et al. 1989). Furthermore, not all insects or their products are conducive to optimal growth and performance of *P. japonica*, as was reported by Guo and Wan (2001) in their comparison of either *Corcyra cephalonica* (Stainton) eggs or trichogrammatid pupae versus *Myzus persicae* (Sulzer) as a food source for laboratory colonies.

Zhang et al. (2007) reported that *P. japonica* larval developmental was significantly prolonged with the addition of olive oil (OO) to the diet and that the addition of 0.3% OO significantly increased larval survival. They also reported that

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the addition of sucrose (S) to the larval diet shortened the preoviposition period in adults (Zhang et al. 2007). Sucrose added to the diet also stimulated larval feeding (Li et al. 2007). Honey and water added to the diet increased the survival rate from larvae to adults in *Harmonia axyridis* (Pallas), but the diet may ultimately reduce the total protein content of the coccinellids (Zhang et al. 2015). Artificial diets containing ground *Tenebrio molitor* (L.) as the primary ingredient increased the larval developmental period, pupal developmental period, and the preoviposition stage, but a slight decrease in developmental uniformity also was reported (Dang and Zhang 2012).

Although artificial diets for predatory insects have been developed, problems with an appropriate *P. japonica* rearing diet remain. For example, *P. japonica* larvae exhibit slowed development when fed with artificial diets (Intazar et al. 2016). Our objective in the study reported herein was to determine the optimal composition of nine ingredients in an artificial diet for rearing *P. japonica*.

Materials and Methods

Our preliminary feeding test results showed that *P. japonica* larvae grow better when fed with *Mythimna separata* (Walker) than fed with *C. cephalonica* eggs, *Antheraea pernyi* (Guerin-Meneville) pupae, or *T. molitor* or *Bactrocera dorsalis* (Hendel) larvae. Therefore, *My. separata* powder was selected as the primary diet ingredient for our feeding test.

Insect culture. *Megoura crassicauda* (Mordvilko) aphids were reared on broad bean (*Vicia faba* L.) plants in rearing cages maintained in the laboratory. The stock colony of *P. japonica* used in this study was initiated from field collections in the spring and summer of 2017 in Qufu, China. *Propylea japonica* adults were fed *Me. crassicauda* aphids in an environmental chamber maintained at 27°C, 72% relative humidity, and 16:8 h light/dark, as described by Guo et al. (2017). *Propylea japonica* individuals used in the study were obtained from these colonies.

Diet preparation. The basic diet (29.42 g in total) used in this study was formulated with nine ingredients (honey [2.5 g], vitamin B [0.02 g], vitamin C [0.04 g], methylparaben [0.03 g], potassium sorbate [0.03 g], agar [0.5 g], Wesson's salt [0.05 g], aphid powder [1.25 g], and water [25 ml]). The aphid powder was prepared by placing *Me. crassicauda* aphids in a thermostatically controlled water bath (50°C) for 6 min; then, they were dried, ground, and stored at –20°C. This basic diet was altered for the study by adding combinations of *My. separata* powder, YE, S, and OO at various concentrations in an orthogonal design comparison. The *My. separata* powder was prepared by freezing third- and fourth-instars at –20°C for 3 h, after which they were ground and stored at –4°C.

Feeding assays. Newly eclosed *P. japonica* larvae were collected and used in the assays. Individual larvae were placed singly in glass tubes (2.5 $\times$ 8 cm, diameter $\times$ height) in which 0.15 cm^3 of the formulated diet had been placed in the bottom of each. A 0.1-cm^3 piece of cotton soaked in 80% (w/v) glucose solution was placed in each tube to maintain moisture. Both the formulated diet and the cotton ball were replaced daily. Tubes were capped with cotton and placed in an environmental chamber as previously described (Yao and Peng 2008). *Propylea japonica* larvae fed with *Me. crassicauda* aphids served as the control treatment.

Larval survival, larval developmental period, pupal developmental period, and adult weight were recorded. Each diet treatment was replicated 3 times with 36 larvae per replicate.

Statistical analyses. The range analysis method, or R method, was used to compare the various treatments in the orthogonal design (Tan et al. 2013). The range analysis method (R method) yields a value, R, that is the difference between the maximum and the minimum of the average sums of indicator data for each factor. The greater the R value, the greater the impact of the tested factor on the dependent variable. Based on R, the order of the impact of the factors was determined. In our study, the four added ingredients of the diets (i.e., insect powder [IP], yeast extract [YE], S, and OO) were the experimental factors, each of which was tested at 3 amounts (IP: 2 g, 4 g, 6 g; YE: 0 g, 1 g, 2 g; S: 0 g, 1 g, 2 g; OO: 0 ml, 0.04 ml, 0.08 ml). SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL) software was used to analyze the data from the orthogonal experimental design and the extreme difference analysis.

Results and Discussion

The $L_9(3)^4$ multi-index orthogonal array design compared mortality, larval duration, pupal duration, and adult weight in response to nine diets (Table 1) and allowed for analysis of the resulting data by the range analysis method (R method). The R values obtained allowed for a relative ranking of individual responses (e.g., mortality, larval duration, pupal duration, and adult weight) to the four additive ingredients and three concentrations of each. According to the R value sizes, the impact on mortality, from greatest to smallest, was YE>S>IP>OO (Table 2). The impacts on larval duration and pupal duration were, from greatest to smallest, IP>YE>OO>S (Table 2). Also, the impact on adult weight was, from greatest to smallest, IP>YE>S>OO (Table 2). An analysis of the impact of the concentrations of each diet additive on the responses provided more in-depth information by identifying an optimal combination of the additives and concentration of each. Thus, the optimal combination of additives, based on mortality and adult weight response, was 6 g IP, 2 g YE, 1 g S, and 0.04 ml OO. The optimal combination of additive levels for larval period duration was 6 g IP, 2 g YE, 2 g S, and 0.08 ml OO, whereas the optimal combination for pupal period duration was 6 g IP, 2 g YE, 1 g S, and 0.08 ml OO.

Addition of the ground *My. separata* (IP) significantly impacted the larval and pupal duration, as well as adult weight. This additive proved to be the most important factor for each response. Therefore, it is considered to be a primary factor with a concentration of 6 g in each of the four responses. YE significantly impacted mortality as the most additive important of the four tested, and it was the second most important factor for larval and pupal duration and adult weight (Table 2). Therefore, it was considered to be a primary factor at a concentration of 2 g for each of the responses. Sucrose had no significant impact on larval or pupal duration (Table 2). However, it significantly impacted mortality (Table 2). Olive oil significantly impacted larval and pupal duration, ranking as the third most important factor for these responses, but it had no significant impact on the other either mortality or adult weight (Table 2).

Table 1. Mean mortality, larval period duration, pupal period duration, and adult weight of *P. japonica* fed nine artificial diets.

Diet	IP* (g)	YE** (g)	S† (g)	OO‡ (ml)	Mortality (%)	Larval Duration (days)	Pupal Duration (days)	Adult Weight (mg)
1	2	0	0	0	53.3	16.0	6.9	3.7
2	2	1	1	0.04	46.7	13.2	5.4	4.0
3	2	2	2	0.08	50.0	11.0	4.6	3.9
4	4	0	1	0.08	60.0	15.1	7.9	3.7
5	4	1	2	0	66.7	17.8	9.6	3.6
6	4	2	0	0.04	40.0	12.2	6.4	4.7
7	6	0	1	0.04	56.7	12.1	4.0	4.0
8	6	1	2	0.08	40.0	10.6	3.4	5.0
9	6	2	0	0	36.7	9.8	3.5	4.7

* IP, insect powder created from macerated and ground *Mythimna separata* bodies (g/29.42 g total diet).
** YE, yeast extract (g/29.42 g total diet).
† S, sucrose (g/29.42 g total diet).
‡ OO, olive oil (ml/29.42 g total diet).

In summary, the optimal diet formula (formula F) of the artificial diet for larvae of *P. japonica* included the following ingredients at the corresponding concentration: *My. separata* powder (6 g), YE (2 g), S (1 g), OO (0.08 ml), honey (2.5 ml), vitamin B (0.02 g), vitamin C (0.04 g), methylparaben (0.03 g), potassium sorbate (0.03 g), aphid powder (1.25 g), agar (0.5 g), Wesson's salt (0.05 g), and water 25 (ml). In assays comparing the optimized formula F diet, this diet was superior to the nine other diet formulas used in the orthogonal design experiment (Table 3). The larval survival rate of *P. japonica* was 72% with formula F, which is higher than that with the other formulas. More importantly, feeding with the optimized formula F could significantly shorten the larval and pupa periods of *P. japonica* (Table 3).

Table 2. R values derived from range analysis method for dietary component impact on development of *P. japonica*.

Dependent Variable	Insect Powder*	Yeast Extract	Sucrose	Olive Oil
Mortality	11.1	14.5	13.4	4.4
Larval duration	4.2	3.4	0.9	2.3
Pupal duration	4.4	1.5	0.5	1.4
Adult weight	0.7	0.6	0.5	0.3

* Insect powder, macerated and ground bodies of *Mythimna separata*.

Table 3. Larval survival and mean (±SE) larval period duration, pupal period duration, and adult weight of *P. japonica* fed different artificial diets.*

Diet	Larval Survival (%)	Larval Duration (days)	Pupal Duration (days)	Adult Weight (mg)
1	46.7	16.00 ± 0.28 d	6.91 ± 0.14 cd	3.70 ± 0.04 d
2	53.3	13.19 ± 0.32 c	5.44 ± 0.17 c	4.00 ± 0.09 cd
3	50.0	11.00 ± 0.22 bc	4.57 ± 0.09 c	3.90 ± 0.04 c
4	40.0	15.13 ± 0.24 cd	7.91 ± 0.19 d	3.70 ± 0.05 c
5	33.3	17.84 ± 0.21 d	9.62 ± 0.33 d	3.60 ± 0.04 d
6	60.0	12.20 ± 0.30 c	6.42 ± 0.10 d	4.70 ± 0.05 b
7	63.3	12.08 ± 0.17 b	3.95 ± 0.18 bc	4.00 ± 0.08 b
8	60.0	10.55 ± 0.23 b	3.38 ± 0.12 b	5.00 ± 0.04 ab
9	63.3	9.80 ± 0.19 ab	3.48 ± 0.13 b	4.70 ± 0.05 b
F**	72.2	8.43 ± 0.15 a	2.50 ± 0.10 a	5.50 ± 0.04 a
Control†	86.1	5.98 ± 0.13 a	1.81 ± 0.09 a	6.90 ± 0.06 a

* Means within a column followed by the same lowercase letter are not significantly different ($P = 0.05$).
** Theoretical optimal diet as determined by the range analysis method.
† Control diet was a basic diet composed of honey, vitamin B, vitamin C, methylparaben, potassium sorbate, agar, Wesson's salt, aphid powder, and water.

The observed lower larval survival rate, longer development period, and smaller adult weight of larvae fed the formula F diet as compared with the control may be due to the lack of a uniform nutrient composition in the larval diets for the different instars. Our results showed that the eclosion rate of *P. japonica* fed the optimized diet formula, including *My. separata* powder, was 72.2 %, which was higher than the eclosion rate when fed a diet including trichogrammatid pupae or *My. persicae* (Guo and Wan 2001). Chen et al. (2011) showed that *P. japonica* larval survival rate was 43.7% when fed on a diet including pig liver, honey, sugar, YE, and OO. The survival rate obtained in our study by using the optimized diet formula F was even higher. Furthermore, the mortality rate was 27.8% when *P. japonica* larvae were fed our optimized diet formula; this was appreciably lower than the mortality rate reported by Liang (2013).

These results show that the formulation screening process developed above was appropriate, and the optimized diet formula likely had a more balanced nutritional structure. The protection and use of predator insects are important aspects of biological control of pest species (Silva et al. 2013). Optimization of the artificial diet can enhance the effect of feeding in *P. japonica* and ensure a satisfactory survival rate of the larvae. This approach should be subject to further improvement, however, with various food sources of *P. japonica* included in future assays and evaluations. For example, egg yolk and insect larvae could shorten the

predator's larval developmental period (Silva et al. 2009), allowing even further optimization of the diet.

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