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Performance of each larval instar and adult of predaceous coccinellid *Coelophora inaequalis* (Fabricius) (Coleoptera, Coccinellidae) fed on natural prey and alternative foods

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Abstract

With the exception of live prey, some lady beetle species consume alternative foods including pollen, nectar and fruit. These alternative foods support the development and reproduction of certains coccinellid species, while others do not benefit as much. The study examined the effects of prey and alternative food sources on the development and reproduction of predaceous coccinellid *Coelophora inaequalis* (Fabricius) (Coleoptera: Coccinellidae), utilizing eggs of rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and bee pollen as alternative foods, while *Aphis gossypii* Glover (Hemiptera: Aphididae) served as the essential prey. Results revealed that 1st instar of *C. inaequalis* larvae fed on *A. gossypii* and *A. gossypii* supplemented with bee pollen success to complete development with no significant differences in growth duration and survival rates (100%) across different developmental stages, including adult longevity and percentage of egg hatch. However, the total number of laid egg was significantly higher on the group that consumed exclusively *A. gossypii*. Results indicated that the switch from natural prey to exclusively alternative prey affects variously each instar larvae and adults of *C. inaequalis*. The 3rd and 4th instars larvae, which have been fed on *A. gossypii* since 1st instar and then switched to exclusively alternative foods failed to progress to the next developmental stage. Only the 2nd instar larvae fed exclusively on bee pollen or rice moth eggs were able to progress to the 3rd and 4th instar. Likewise, male and female adults of *C. inaequalis* survived for a temporarily time when exclusively fed on bee pollen or rice moth eggs.

Key Words

Aphis gossypii, bee pollen, development, longevity, rice moth

Introduction

Lady beetles (Coleoptera: Coccinellidae) are major natural enemies of aphids throughout their life cycles. Naturally, lady beetles are generalist predators consume various small preys such as aphids, psyllids, mealybugs and scale insects in their habitats. Moreover, many reports indicated that with the exception of live prey, some lady beetle species consume alternative foods including pollen, extrafloral nectar, foliage and fruit (Pemberton and Vandenberg 1993; Koch et al. 2004; Moser et al. 2008; He and Sigsgaard 2019). One of the most nutritious of non-prey foods consumed by coccinellid is pollen. Lundgren (2009) reported that pollen is quantitatively superior to prey in terms of energy, protein and carbohydrates, and is differed significantly in lipids content compared with prey. In addition, pollen also contains other beneficial nutrients needed for insects such as amino acids, sugars, sterols, vitamins, flavonoids, carotenoids, and minerals (Atrouse et al. 2004; Lundgren and Wiedenmann 2004). At least 39 species of predatory lady beetle have been recorded as consuming pollen (Lundgren 2009). Previous studies reported both beneficial and unbeneficial roles of pollen on the development and reproduction of coccinellid species. For certain coccinellid species, consuming pollen alongside aphids can enhance reproduction and overall performance (Triltsch 1999; De Clercq et al. 2005; Omkar 2006). Larvae of *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae) fed only on pollen of Rosaceae successfully develop to adults (Hemptinne and Desprets 1986). A diet of bee pollen alone allowed 35–48% of the larvae of the field population of multicoloured Asian lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), exotic species settle down in Europe, successfully reach adulthood and females able to produce a small number of viable eggs (Berkvens et al. 2008). The exploitation pollen as a food to sustain development and reproduction in the absence of insect of *H. axyridis* supported competitive advantage over the native European lady beetles (Berkvens et al. 2008). Likewise, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) can develop and reproduce on a diet consisting solely of maize pollen (Lundgren and Wiedenmann 2004). In contrast, Xia et al. (2024) reported that pollen did not support the development time of immature stages of *H. axyridis* reared on canola pollen together with aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) when compared with other group fed only on *M. persicae*. Moreover, female fecundity of *H. axyridis* reared on mix diet were significantly lower than that of only fed *M. persicae* and this phenomenon found in *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) also. Likewise, bee pollen did not support to produce mature oocytes of predatory lady beetle *Brumoides foudraii* (Mulsant) (Coleoptera: Coccinellidae) reared on the mix of mealybug *Ferrisia dasylirii* (Cockerell) (Hemiptera: Pseudococcidae) and pollen when compared to other group only fed on *F. dasylirii* (Lima et al. 2020). Besides this, eggs of the rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) served as an efficient host for mass-rearing various species of parasitoids and predators (Manjunath 2023). Ali (2023) studied on the suitable as alternative food for coccinellids rearing and reported the survival rates and predatory efficacy of the *Serangium japonicum* Chapin (Coleoptera: Coccinellidae) are similar to those feeding on natural host *Bemesia tabaci* Gennadius (Hemiptera: Aleyrodidae) when *S. japanicum* larvae from 2^{nd} larval instar onwards reared on *C. cephalonica* eggs.

Coelophora inaequalis (Fabricius) (Coleoptera: Coccinellidae) is widely distributed across India (Andamans), Sri Lanka, Southeast Asia (Thailand, Malaysia, Indonesia, Philippines), New Guinea, New Caledonia, Micronesia and Australia (Poorani 2023). *Coelophora inaequalis* was introduced to Hawaii and Florida as a biological control agent to control the yellow sugarcane aphid *Sipha flava* (Forbes) (Hemiptera: Aphididae) (Peck and Thomas 1998). Recently, *C. inaequalis* are important lady beetle species commonly found in many regions of Thailand distributed cover Northern to Southern. Biology of *C. inaequalis* settled down in Thailand has not yet been studied. Previous studies of the life history of *C. inaequalis* reviewed that *C. inaequalis* fed on *Aphis craccivora* Koch (Hemiptera: Aphididae) has the total development period, including incubation, was 10–11 days. The life span of the female adults was 112–126, while that of the male adults was 111–126 days and the total consumption from egg hatching to adult death was 2,821–3,509 prey individuals for male and 2,959–4,779 prey individuals for female. The number of eggs laid by the female total 510–783 eggs for a period of 30 days (Mora et al. 1995). Understanding the biology and possibility of mass rearing of *C. inaequalis* can be useful for biological control of insect pests and contribute to sustainable agricultural practices.

Methods

Mass rearing of *Corcyra cephalonica*

Eggs of rice moth *C. cephalonica* supported by Surat Thani Agricultural Technology Promotion Center (Plant Protection), Surat Thani, Thailand were sprinkled on broken rice as 0.1 g/1 kg broken rice kept in 20 × 30 × 10 cm. Larvae of *C. cephalonica* were reared on broken rice under 25 ± 1 °C, 70 ± 5% RH, and 12 hours photoperiod in the laboratory. After *C. cephalonica* larvae developed to adult, male and female of *C. cephalonica* were moved to net bags and put on black plastic cup for egg harvesting. Harvested rice moth eggs were used to carry out the experiment.

Mass rearing of *Coelophora inaequalis*

Adults of *C. inaequalis* were collected from organic vegetable farm, Paron Subdistrict, Kanchanadit District, Surat Thani Province, Thailand. Each pair of male and female adults were reared on *Aphis gossypii* Glover (Hemiptera: Aphididae) ad libitum in 6 × 4.5 × 3 cm plastic box under 25 ± 1 °C, 70 ± 5% RH, and 12 hours photoperiod in the laboratory. Water was supplied using moistened cotton ball put on 80 ml plastic cup. After female laid eggs, male and female of *C. inaequalis* were moved to new 6 × 4.5 × 3 cm plastic box for protection of egg cannibalism. Early hatched *C. inaequalis* larvae were used in this study.

Experiment: Effect of prey and alternative food sources on *C. inaequalis* development and the ability of larvae to adapt to new food sources

First instar larvae of *C. inaequalis* were released in 6 × 4.5 × 3 cm plastic box and fed on tested foods: 1^{st}-2^{nd} instar of *A. gossypii* (natural prey), 1^{st}-2^{nd} instar of *A. gossypii* with bee pollen (natural prey with alternative food), bee pollen (alternative food) and rice moth eggs (alternative food). Previous observation found that 1^{st} instar larvae of *C. inaequalis* only consumed *A. gossypii* and ignored rice moth eggs when two preys were offered simultaneously. Then, *A. gossypii* with rice moth eggs treatment was cut out from the experiment. Fifteen larvae were used in each foods. Foods were supplied to *C. inaequalis* larvae ad libitum. Water was provided to larvae as same as adults under 25 ± 1 °C, 70 ± 5% RH, and 12 hours photoperiod in the laboratory. Developmental period and the number of consumed prey of each *C. inaequalis* stage and adult longevity were observed. The number of laid egg through female's lifespans and percent of egg hatch were recorded. In addition, the 2nd, 3rd, 4th instars larvae, male and female adults of *C. inaequalis*, which have been fed on *A. gossypii* since first instar, were tested with the alternative foods: rice moth eggs and bee pollen. All other experimental conditions were as for the test that starts from the 1st instar larvae.

Data analysis

One-way ANOVA or t-test were used to test the effect of foods on *C. inaequalis* development. Response variables analyzed were the duration of larva-to-adult period, the number of consumed prey, adult longevity, the number of egg laid by female throughout their lifespan and percent of egg hatch. Post-hoc, pairwise comparisons of means were made using Tukey tests. The data were analyzed using SPSS statistics 17.0.

Results

Effect of prey and alternative food sources on *C. inaequalis* development

Foods effected the development and survival of *C. inaequalis*. The results indicated that only the larvae fed on *A. gossypii* and a combination of *A. gossypii* with bee pollen were able to grow into adults with no significant differences in growth duration and survival rates (100%) across different developmental stages, including adult longevity (Table 1). The research indicates that when *C. inaequalis* females fed only on *A. gossypii*, they produced significantly more eggs compared to females fed on *A. gossypii* supplemented with bee pollen while the egg hatch percentage did not differ among two groups (Table 1).

First instar larvae of *C. inaequalis* exclusively fed on bee pollen or rice moth eggs died on first or second days of the experiment. When comparing the 2nd larvae, it was found that *C. inaequalis* larvae fed on *A. gossypii* from the 1st instar and then switched to exclusively bee pollen in the 2nd instar had a developmental duration in the 2nd instar that was not significantly different from those larvae fed solely on *A. gossypii* or a combination of *A. gossypii* and bee pollen (Table 1). These larvae were also able to survive into the 3rd instar (100%). In contrast, *C. inaequalis* larvae that were fed only rice moth eggs upon entering the 2nd instar exhibited a significantly longer developmental duration from the 2nd to the 3rd instar compared to the other groups, along with a lower survival rate (33.33%) (Table 1). When comparing the growth of *C. inaequalis* larvae in the 3rd instar, it was found that larvae fed solely on bee pollen from the 2nd instar took significantly longer to develop from the 3rd to the 4th instar than those larvae fed on *A. gossypii* or those fed a combination of *A. gossypii* and bee pollen. Additionally, the survival rate into the 4th instar was lower for the larvae fed exclusively on bee pollen (40%). Meanwhile, larvae fed exclusively on rice moth eggs from the 2nd instar can survive for a period in the 3rd instar with low survival rate (26.67%) and they were unable to develop into the 4th instar (Table 1).

The results indicated that *C. inaequalis* larvae that were initially fed on *A. gossypii* during the 1st instar and subsequently switched to a diet of exclusively bee pollen or rice moth eggs in the 3rd or 4th instar failed to progress to the next developmental stage. They could survive for 3–4 days before died (Table 1). The lifespan of male and female adults that were fed on *A. gossypii* from the 1st

Table 1 Developmental period, survival rate and reproduction of *C. inaequalis* in each stage fed on different foods.

Foods	Developmental stage					total	Adult longevity		No. laid egg / egg hatch (%)
	1st instar	2nd instar	3rd instar	4th instar	pupa		Male	Female	
A. gossypii	1.13 ± 0.09	2.20 ± 0.14b	2.13 ± 0.09c	2.40 ± 0.13b	4.07 ± 0.07	11.93 ± 0.25	103.78 ± 2.07[5]a	110.83 ± 2.24[2]a	308.33 ± 7.95[2] /82.67 ± 2.58[2]
A. gossypii+bee pollen	1.20 ± 0.11	2.33 ± 0.13b	2.27 ± 0.12c	2.53 ± 0.13b	4.13 ± 0.09	12.47 ± 0.26	101.63 ± 1.74[4]a	110.14 ± 2.20[3]a	292.33 ± 5.00[3] /81.33 ± 1.58[3]
bee pollen (1st inst.)	1.20 ± 0.11	-	-	-	-	-	-	-	-
bee pollen (2nd inst.)	-	1.00 ± 0.00b	4.17 ± 0.17a	1.83 ± 0.17[2]c	-	-	-	-	-
bee pollen (3rd inst.)	-	-	3.33 ± 0.13b	-	-	-	-	-	-
bee pollen (4th inst.)	-	-	-	3.40 ± 0.13a	-	-	-	-	-
bee pollen (adult)	-	-	-	-	-	-	8.80 ± 0.40b	9.13 ± 0.40b	-
Rice moth egg (1st inst.)	1.00 ± 0.00	-	-	-	-	-	-	-	-
Rice moth egg (2nd inst.)	-	3.80 ± 0.20a	5.00 ± 0.00[1]a	-	-	-	-	-	-
Rice moth egg (3rd inst.)	-	-	3.33 ± 0.13b	-	-	-	-	-	-
Rice moth egg (4th inst.)	-	-	-	3.20 ± 0.11a					
Rice moth egg (adult)	-	-	-	-	-	-	4.00 ± 0.24c	3.93 ± 0.21c	-
Statistical analysis	$F_{3,56}$ = 1.143 P = 0.340	$F_{3,56}$ = 21.554 P < 0.0001	$F_{5,73}$ = 73.072 P < 0.0001	$F_{4,61}$ = 18.801 P < 0.0001	t = -0.592 P = 0.559	t = -1.497 P = 0.146	$F_{3.43}$ = 1373.770 P < 0.0001	$F_{3.39}$ = 1260.277 P < 0.0001	t = 2.497 P = 0.032; t = 1.703 P = 0.119

Number of replicate = 15. [1,2,3,4,5] were 4, 6, 7, 8 and 9 replicates, respectively. Values (mean ± SE) in the same column followed by the different letter are statistically different (Tukey-test, P < 0.05). Significance is based on transformed data using log (x + 1), non-transformed data are presented.

instar to 4th instars and then switched to exclusively bee pollen or rice moth eggs after adult emergence was significantly shorter than that of groups fed on *A. gossypii* and a combination of *A. gossypii* with bee pollen (Table 1). Bee pollen and rice moth eggs could support the survival of male and female adults of *C. inaequalis* for 8–9 days and 3–4 days, respectively (Table 1).

The study results clearly indicate that natural food such as *A. gossypii*, or a combination of *A. gossypii* and bee pollen, can serve as food for the 1st instar larvae, allowing them to grow into adults. For alternative foods, bee pollen can support the 2nd instar larvae in growing into 3rd and 4th instars, although they cannot develop into the pupal stage. Meanwhile, rice moth eggs can be used as food for the 2nd instar to grow into 3rd instar, but they cannot progress from 3rd to 4th instars (Fig. 1). In addition, bee pollen and rice moth eggs alone were insufficient for the development of 1st, 3rd and 4th instar larvae to the next stage.

Results presented that with the exception of 2nd instar larvae (t-test: $t = 2.361$, $P = 0.028$), there was no the significant different in the number of consumed prey among different diet of each larval stage (Fig, 2). Larvae of *C. inaequalis* that were exclusively fed on aphids consumed a greater quantity of aphids than those larvae that were fed a combination of aphids and bee pollen, both at the 1st, 2nd, and 4th instars, as well as in total consumption across all instars. However, *C. inaequalis* larvae that were given aphids along with bee pollen consumed higher aphid number in 3rd instar (t-test: 1st instar $t = 0.598$, $P = 0.554$; 3rd instar $t = -1.126$, $P = 0.270$; 4th instar $t = 0.726$, $P = 0.475$; total $t = 1.537$, $P = 0.135$) (Fig. 2).

Discussion

Previous researches indicated that some important lady beetle species used for biological control survived and reproduced when reared on non-natural preys (pollen, nectar, eggs, larvae and pupae of other insects, etc.) (Pemberton and Vandenberg 1993; Koch et al. 2004; Moser et al. 2008; Lundgren 2009). However, results of this study presented that 1st, 3rd and 4th instar larvae of *C. inaequalis* fed only on bee pollen or rice moth eggs could not survived to the next developmental stage. The inability of each instar larvae to survive and continue developing when fed solely on rice moth eggs or bee pollen may be explained by different factors. For 1st instar larvae, physiological limitations are involved, as their mandibles are not yet sufficiently strong to bite or grind food with a relatively hard texture into smaller pieces for consumption. Then, we hypothesize that the rice moth eggs and bee pollen are too tough for the 1st instar larvae of *C. inaequalis* to consume. The limited physiological capabilities of coccinellid larvae for prey consumption was also reported by Michaud (2005). While the reason that 3rd and 4th instar larvae are unable to continue developing may stem from a change in diet, it indicates that the larvae of *C. inaequalis* are unable to adapt to the new food, which is an alternative diet, after being fed exclusively on aphids until reaching the 3rd and 4th instar. Interestingly, 2nd instar larvae fed on *A. gossypii* from the 1st instar and then switched to exclusively rice moth eggs or bee pollen in the 2nd instar can develop to 3rd and 4th instar when fed exclusively on rice moth eggs and bee pollen, respectively. We hypothesize this phenomenon that 2nd instar larvae had more mandible strength than 1st instar larvae

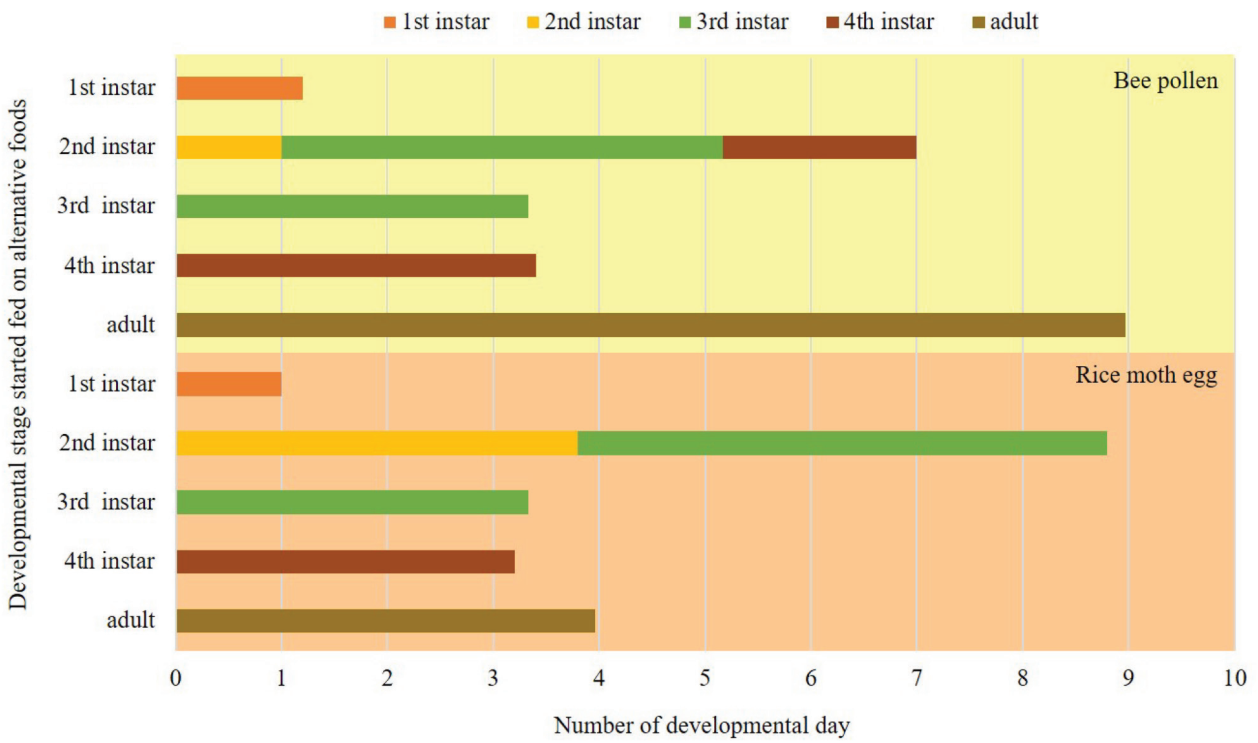

Figure 1. The development of *C. inaequalis* in each developmental stages started fed on alternative foods (bee pollen and rice moth egg data were yellow and orange zones, respectively).

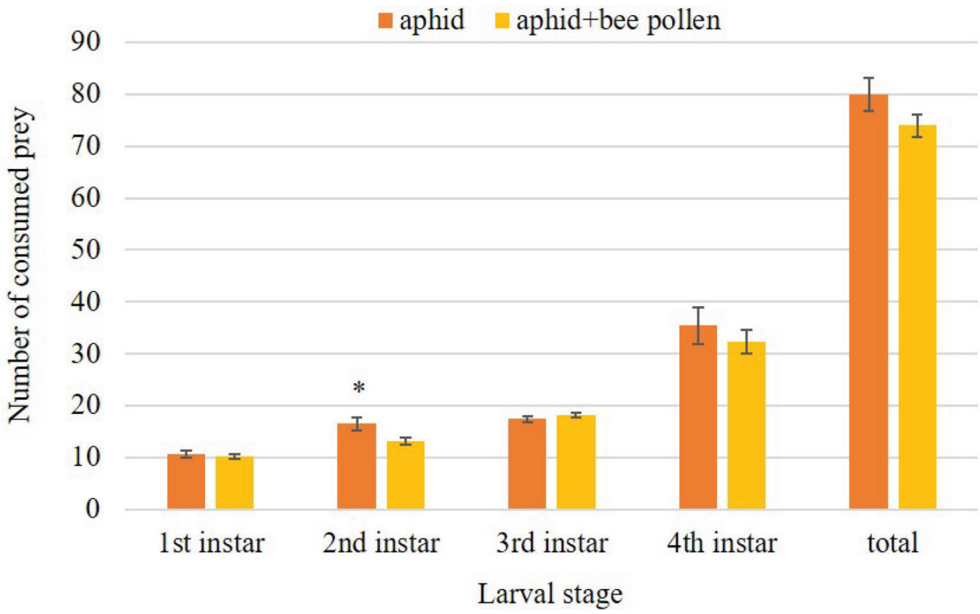

Figure 2. Mean number (±SE) of prey consumed by *C. inaequalis* of each larval stages. Statistical significance different among treatments presented with one asterisk (Tukey test: $P < 0.05$).

and possessed a more adaptable digestive system that can better accommodate alternative food sources compared to 3rd and 4th instar larvae. Consequently, the transition from a diet exclusively consisting of aphids to alternative food sources facilitates a greater degree of adaptability in 2nd instar larvae than in 3rd and 4th instar larvae. Previous study also indicated the unacceptable on rice moth eggs of the 1st instar larvae of *S. japonicum* (Ding et al. 2019) but *S. japanicum* larvae from 2nd larval instar could consume rice moth eggs and completely develop to adults (Michaud 2005). The results of this study indicated that while only the 2nd instar larvae fed exclusively on bee pollen or rice moth eggs were able to progress to the next developmental stage, both bee pollen and rice moth eggs were sufficient to sustain the survival of 1st, 3rd, and 4th instar larvae, as well as *C. inaequalis* adults, for a temporary period.

In addition, result of this study indicated that *C. inaequalis* larvae reared on *A. gossypii* with bee pollen tend to consumed *A. gossypii* fewer than that of *C. inaequalis* fed on *A. gossypii* alone. This phenomenon was found in *H. axyridis* and *P. japonica* with decreasing in predation when pollen was provide as additional food (Xia et al. 2024). They suggested that when pollen is readily available, *H. axyridis* and *P. japonica* may not need to rely as heavily on predation to obtain food. This can lead to a decrease in the number of insects they consume. Although *C. inaequalis* reduced the consumption of *A. gossypii*, longevity of male and female adult did not differ between the group that consumed exclusively *A. gossypii* and the group that consumed *A. gossypii* supplemented with bee pollen. Although *C. inaequalis* could complete their developments and reproduce successfully when feeding on *A. gossypii* or *A. gossypii* supplemented with bee pollen, the total number of laid egg was significantly higher on the group that consumed exclusively *A. gossypii*. This suggests that *C. inaequalis* has specific nutritional requirements from *A. gossypii* to produce eggs or support its reproductive system, which are nutrients that cannot be fulfilled by bee pollen. This behavior of *C. inaequalis* was partially explained by nutritionally poor satiety concept which satiety, defined as the means by which further eating is inhibited, hunger decreases, and fullness increases after a meal (Blundell et al. 2010), may play a role in altering the amount of food and energy consumed by an individual (Health Canada, 2012). In animals, a nutritionally poor diet leads to poor satiety (feeling full) because it lacks the essential nutrients (like protein, fiber, fats). Numerous studies indicate that bee pollen is primarily composed of carbohydrates, with protein and lipids also present; essential micronutrients, including minerals and vitamins, are found as well (da Silva et al. 2014; Thakur and Nanda 2020; Végh et al. 2021; Lau et al. 2022). However, bee pollen was found to contain pesticide residues also (Haroune et al. 2015; Friedle et al. 2021). Consequently, consuming bee pollen alongside aphids may satisfy *C. inaequalis*s' hunger but results in a lower intake of essential nutrients required for egg development. Furthermore, this may partly be attributed to the side effects of pesticide possibly contaminants present in the bee pollen used in this experiment. Michaud (2000) indicated that coccinellids need to consume more nutrients than required for maintenance to reproduce. Aphids are the natural preys of *C. inaequalis* that support their success on development and reproduction (Mora et al. 1995). In addition, results of previous studies indicated that pollen or bee pollen did not support the reproduction of all coccinellid species but generally they can prolong larval and adult longevity when prey are absent (Seagraves 2009; Lima et al. 2020). Xia et al. (2024) and Seagraves (2009) proposed that pollen from specific plant species might contain secondary metabolites that are toxic to insects or that inhibit their oviposition. Hence, result of this study also confirmed that bee pollen did not increase the fecundity of *C. inaequalis*. For the Coccinellidae as a group, *A. gossypii* has been proposed to classify prey types as "essential" because they support both completed larval development and successful adult reproduction, and "alternative" for rice moth eggs as they serve only as an energy source to extend larva and adult longevity (Hodek and Honek 1996). This study found some interesting result about performance of 2nd larval instar of *C. inaequalis* on the switch of natural prey to alternative foods. Other than the development of artificial diets, further research on the mass rearing of *C. inaequalis* may focus on the potential for enhancing their performance by alternating between natural prey and artificial diets.

Conclusions

Bee pollen and rice moth eggs serve as alternative prey for *C. inaequalis*, providing temporary sustenance. Although *C. inaequalis* shows a greater acceptance of bee pollen as a food source compared to rice moth eggs, *C. inaequalis* adults that were fed on *A. gossypii* supplemented with bee pollen exhibited a decreased in fecundity, compared to those fed solely on *A. gossypii*. Interestingly, the 2nd instar larvae of *C. inaequalis* exhibited a greater acceptance of bee pollen and rice moth eggs compared to both the 1st, 3rd and 4th instar larvae, which facilitated their development into higher instars. Bee pollen and rice moth eggs could sustain *C. inaequalis* adults survive for a period of approximately 8–9 days and 3–4 days, respectively. This presents an intriguing opportunity for further research into the development of artificial foods for the mass rearing of *C. inaequalis* and the suitable method of using natural prey alternated with artificial food in the rearing of *C. inaequalis* and other predaceous coccinellid species.

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