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Effect of Prey and Alternative Food Sources on Development and Reproduction of *Coelophora inaequalis* Fabricius (Coleoptera: Coccinellidae)

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Simple Summary

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 aim of this study is to investigate effects of pollen and eggs of rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) on development and reproduction of the predatory lady beetle *Coelophora inaequalis* (Fabricius) (Coleoptera: Coccinellidae). This study reveals that 1st instar larvae of *C. inaequalis* cannot survive when exclusively reared on bee pollen or rice moth eggs. In contrast, these larvae can develop into adults when fed on *Aphis gossypii* Glover (Hemiptera: Aphididae) or *A. gossypii* supplemented with bee pollen. In spite of the addition of bee pollen leads to a shorter development time for *C. inaequalis*, a lower survival and fecundity rate were observed in the group fed on *A. gossypii* with bee pollen. Moreover, compared to the group fed exclusively on *A. gossypii*, those consuming *A. gossypii* supplemented with bee pollen exhibited shorter adult longevity. Adults of *C. inaequalis* can survive for 4–9 days on a diet consisting solely of bee pollen, rice moth eggs, or a combination of both.

Abstract

Lady beetle can utilize the alternative food sources to enhance their diets, other than essential prey. These alternative diets may enhance their developments, survival and fecundity. The study examined the effects of prey and alternative food sources on the development and performance of predaceous coccinellid *Coelophora inaequalis* (Fabricius) (Coleoptera: Coccinellidae), utilizing eggs of rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) and bee pollen as alternative diets, while *Aphis gossypii* Glover (Hemiptera: Aphididae) served as the essential prey. Results revealed that 1st instar larvae of *C. inaequalis* are not able to maintain their development by feeding exclusively on an alternative diets, while other groups fed on *A. gossypii* and *A. gossypii* supplemented with bee pollen success to complete development. Although *C. inaequalis* that fed on *A. gossypii* supplemented with bee pollen experienced a shorter development time than those fed solely on *A. gossypii*, they exhibited decreased fecundity, lower survival rate and shorter adult longevity compared to the latter group. Adult of *C. inaequalis* fed exclusively on rice moth eggs or bee pollen or a combination of both can survive for 4-9 days. Rice moth eggs and bee pollen can serve as alternative food sources to enhance longevity of *C. inaequalis* adults.

Keywords: bee pollen; rice moth; *Aphis gossypii*

1. Introduction

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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 [1–4]. One of the msost nutritious of non-prey foods consumed by coccinellid is pollen. [4] 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 [5–6]. At least 39 species of predatory lady beetle have been recorded as consuming pollen [4]. 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 [7-9]. Larvae of *Adalia bipunctata* (Coleoptera: Coccinellidae) fed only on pollen of Rosaceae successfully develop to adults [10]. 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 [11]. 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 [11]. In contrast, [12] 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* [13].

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 [14] were studied on the suitable as alternative food for coccinellids rearing. [15] 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 2nd 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 [16]. *Coelophora inaequalis* was introduced to Hawaii and Florida as a biological control agent to control the yellow sugarcane aphid *Sipha flava* (Forbes) (Hemiptera: Aphididae) [17]. 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 [18]. 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.

2. Materials and Methods

2.1. 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 diets composed of broken rice, rice bran and sugar (1: 1: 0.1, W:W). The ratio of rice moth eggs and diet was 0.1 g/1 kg of diet and

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kept in 20×30×10 cm plastic box 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.

2.2. 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.

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

First instar larvae of *C. inaequalis* were released in 6×4.5×3 cm plastic box and fed on tested diets: rice moth eggs, bee pollen, *A. gossypii* with bee pollen, and only *A. gossypii* as control. Previous observation found that 1st 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. Twenty larvae were used in each diets, and three replicates were established. Diets 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.

Experiment II Effect of alternative food sources on longevity of *C. inaequalis* adult

To determine the effect of prey and alternative food sources on performance of *C. inaequalis* adult, newly emerged male and female *C. inaequalis* adults were fed on three different diets: rice moth eggs, bee pollen and rice moth eggs with bee pollen. Twenty male and female adults were fed per diet, and three replicates were established. All other experimental conditions were as for the experiment I. Adult longevity were observed.

2.3. Data Analysis

Two-way ANOVA was used to test for effect of *C. inaequalis* groups of each replicate, diet, and their interactions. If there was no significant interaction effect then the dependent data were pooled across individual group of each repeated experiment times (replicate) and effect of diet was tested using one-way ANOVA or t-test analysis. If a significant interaction effect was detected, then the effect of diet on the dependent data was analyzed by one-way ANOVA or t-test for each repeated experiment times. Data were transformed, if required, to meet the assumptions of ANOVA or t-test, and then back-transformed for graphical presentation of the number of prey and for the development time table. 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.

3. Results

3.1. Effect of Prey and Alternative Food Sources on C. inaequalis Development and Reproduction

Diets effected the development and survival of *C. inaequalis*. First instar larvae of *C. inaequalis* did not consume bee pollen and rice moth eggs, and all of larvae died on second days of the experiment. Then, *C. inaequalis* larvae fed on *A. gossypii* with bee pollen, and *A. gossypii* alone were observed their developments. There was no significant interaction effect between *C. inaequalis* groups of each repeated experiment times and diets in the developmental periods of *C. inaequalis* (ANOVA: 1^{st} instar $F_{2, 114} = 0.400$, $P = 0.671$; 2^{nd} instar $F_{2, 114} = 0.282$, $P = 0.754$; 3^{rd} $F_{2, 114} = 0.049$, $P = 0.952$; 4^{th} instar $F_{2, 114} = 0.337$, $P = 0.715$; pupal stage $F_{2, 103} = 1.059$, $P = 0.351$; adult stage; $F_{2, 93} = 0.302$, $P = 0.740$; male

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adult $F_{2, 39} = 0.497$, $P = 0.612$; female adult $F_{2, 48} = 1.762$, $P = 0.183$), so data were pooled across replicates. Results presented that with the exception of 3rd larval instar, *C. inaequalis* fed on *A. gossypii* supplemented with bee pollen seemed to develop in each stage faster than that of *C. inaequalis* fed on *A. gossypii* alone (Table 1). The higher mortality rate occurred on *C. inaequalis* fed on *A. gossypii* aphids with bee pollen as a supplementary food source. Finally, the number of survivable adult and adult longevity of *C. inaequalis* fed exclusively on *A. gossypii* was higher than that of *C. inaequalis* group fed on *A. gossypii* supplemented with bee pollen (Table 1). There was no significant difference in male longevity between the two diets. Females, on the other hand, demonstrate significantly longer lifespans when fed exclusively on *A. gossypii*. (Table 1).

There were no significant interaction effect between *C. inaequalis* groups of each repeated experiment times and diets in the number of laid eggs and percentage of egg hatch (ANOVA: laid eggs $F_{2, 41} = 0.089$, $P = 0.915$; percentage of egg hatch $F_{2, 41} = 0.086$, $P = 0.918$), then data were pooled across repeated experiment times. The research indicates that when *C. inaequalis* females fed only on *A. gossypii*, they produced significantly more eggs and a higher percentage of those eggs hatched compared to females fed on *A. gossypii* supplemented with bee pollen (t-test: laid eggs $t = 6.815$, $P < 0.0001$; percentage of egg hatch $t = 6.031$, $P < 0.0001$). Specifically, *C. inaequalis* females fed on *A. gossypii* alone laid 349.46±16.86 eggs with an 80.83±1.51% hatch rate, while those with the pollen supplement laid 214.10±8.96 eggs and had a 62.87±2.42% hatch rate.

Table 1. Developmental period of *C. inaequalis* fed on different diets.

Life stage	**Development time (days, Mean±SE)**		**t-test**
	A. gossypii	***A. gossypii* + bee pollen**	
1st larva	3.68±0.09	3.20±0.05	t = -4.634, P<0.0001
n	60	60	
2nd larva	2.87±0.11	2.67±0.09	t = -1.289, P = 0.200
n	60	60	
3rd larva	2.15±0.05	2.27±0.06	t = 1.577, P = 0.118
n	60	60	
4th larva	2.98±0.07	2.60±0.06	t = -3.820, P<0.0001
n	60	60	
pupa	4.53±0.10	4.13±0.34	t = -3.584, P = 0.001
n	57	52	
Overall immature	16.26±0.18	14.98±0.11	t = 6.046, P<0.0001
n	57	52	
Adult	108.04±0.87	104.91±0.86	t = 2.497, P=0.014
n	53	46	
Male adult	103.88±1.18	102.70±0.10	t = 0.719, P = 0.476
n	25	20	
Female adult	111.75±0.76	106.62±1.24	t = 3.560, P = 0.001
n	28	26	

n = number of C. inaequalis.

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There was no significant interaction effect between *C. inaequalis* groups of each repeated experiment times and diets in the number of prey consumed by *C. inaequalis* (ANOVA: 1st instar $F_{2, 114}$ = 0.164, P = 0.849; 2nd instar $F_{2, 114}$ = 0.132, P = 0.877; 3rd $F_{2, 114}$ = 0.688, P = 0.504; 4th instar $F_{2, 114}$ = 0.352, P = 0.704), so data were pooled across experiment times. Results presented that with the exception of 1st instar larvae (t-test: t = 0.728, P = 0.468), there was the significant different in the number of consumed prey among different diet of each larval stage (Figure 1). The number of consumed *A. gossypii* was higher in *C. inaequalis* fed only on *A. gossypii* than that of *C. inaequalis* fed on *A. gossypii* supplemented with bee pollen in 2nd and 4th instar larvae and also all larval stages (t-test: 2nd instar t = 15.923, P < 0.0001; 3rd instar t = -2.851, P = 0.005; 4th instar t = 9.098, P < 0.0001; total t = 8.115, P < 0.0001) (Figure 1).

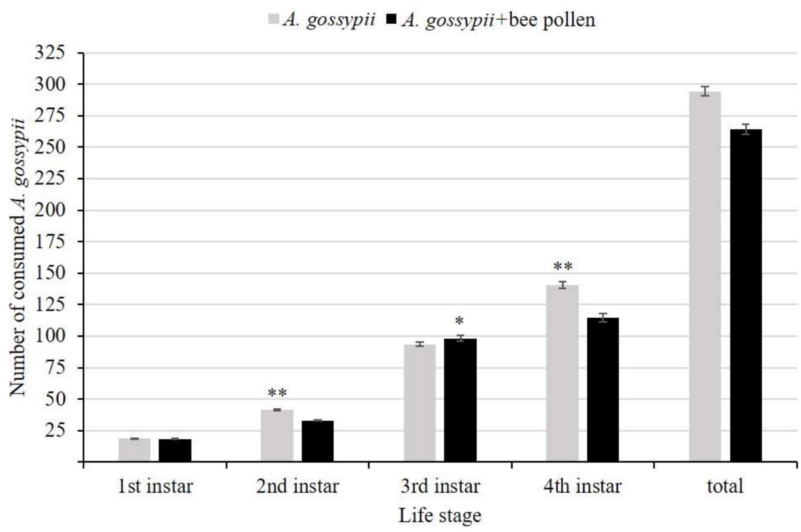

Figure 1. Mean number (±SE) of prey consumed by *C. inaequalis* of each larval stages. Statistical significance different among treatments presented with one asterisk (Tukey test: P < 0.05) or two asterisks (Tukey test: P < 0.0001). Significance is based on log (x + 1)-transformed data, non-transformed data are plotted.

3.2. Effect of Alternative Food Sources on Longevity of C. inaequalis Adult

Results indicated that there were no significant interaction effect between *C. inaequalis* groups of both sexes of each repeated experiment times and diets among the longevity period of *C. inaequalis* male and female adults (ANOVA: male $F_{4,171}$ = 2.163, P = 0.075; female $F_{4,171}$ = 1.332, P = 0.260), then data were pooled across experiment time. The lifespan of male and female adults that were exclusively fed rice moth eggs was significantly shorter than that of groups fed bee pollen or a combination of rice moth eggs and bee pollen (Table 2). Notably, all females on these alternative diets did not lay any eggs throughout their lives.

Table 2. The longevity of *C. inaequalis* adults fed on different diets.

Prey	Longevity (day)	
	male	female
Bee pollen	8.88±0.27a	9.38±0.27a
Rice moth egg	4.45±0.16b	4.05±0.12b
Rice moth egg + bee pollen	8.84±0.26a	9.34±0.23a
F-test	$F_{2,177}$ = 237.392, P < 0.0001	$F_{2,177}$ = 448.443, P < 0.0001

Values (mean ± SE) in the same column followed by a different letter are statistically different based on Tukey-test for longevity of each sexes (60 individuals of each diets) at P < 0.05. Significance is based on log (x + 1)-transformed data, non-transformed data are presented.

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4. 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.) [1–4]. However, results of this study presented that *C. inaequalis* larvae fed only on bee pollen or rice moth eggs could not survived from 1st instar to 2nd instar stage.

Interestingly, *C. inaequalis* adult could survived for 4-5 days and 8-9 days even only consumed rice moth eggs and bee pollen, respectively but did not lay eggs during that time. These results are partially explained by the different of prey suitability for larval and adult coccinellids. [19] noted that adult coccinellids possess stronger mandibles and more advanced digestive systems compared to their larval counterparts. Consequently, *C. inaequalis* larvae tend to reject non-prey foods due to their limited physiological capabilities. In this study, the 1st instar larvae of *C. inaequalis* were unable to survive when exclusively fed bee pollen or rice moth eggs, exhibiting the unacceptable behavior of not consuming them. Previous study also indicated the unacceptable on rice moth eggs of the 1st instar larvae of *S. japonicum* [20] but *S. japanicum* larvae from 2nd larval instar could consume rice moth eggs and completely develop to adults [19]. Although *C. inaequalis* larvae reared on *A. gossypii* supplemented with bee pollen had shorter development time, the survival rate was lower than that of *C. inaequalis* groups fed on *A. gossypii* solely. Thus, bee pollen may give some beneficial nutrients that support the development of *C. inaequalis*. In addition, result of experiment I indicated that *C. inaequalis* larvae reared on *A. gossypii* supplemented 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 [12]. 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. The reduced consumption of *A. gossypii* by *C. inaequalis* larvae results in insufficient nutrient intake for their performance, leading to a higher mortality rate compared to the group that was exclusively fed *A. gossypii*. For results of diets tests in adults, bee pollen and rice moth eggs may provide essential nutrients for *C. inaequalis* adults but they did not lay eggs during their lifespans, regardless of these food sources. This implies that the *C. inaequalis* adults require specific food sources for survival but do not reproduce when given these limited diets. This suggests that *C. inaequalis* has specific nutritional requirements for reproduction that neither rice moth eggs nor bee pollen can fulfill. This could be due to a lack of certain essential nutrients, insufficient quantities of necessary nutrients, or a combination of both as [21] indicated that coccinellids need to consume more nutrients than required for maintenance to reproduce. For example, [22] demonstrated that adult female *H. axyridis* were unable to produce viable eggs when fed exclusively on a diet of *Aphis spiraecola* Patch (Hemiptera: Aphididae), despite the fact that this aphid species can support the complete development of larvae into adults. In this study, *C. inaequalis* could complete their developments and reproduce successfully when feeding on *A. gossypii* or *A. gossypii* supplemented with bee pollen. Aphids are the natural preys of *C. inaequalis* that support their success on development and reproduction [18]. Nevertheless, the fecundity of *C. inaequalis* adults fed on *A. gossypii* supplemented with bee pollen was significantly lower than that of other group fed on *A. gossypii* alone. 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 [13,23]. [12,23] 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, it has been proposed to classify prey types as “essential” if they support both completed larval development and successful adult reproduction, or “alternative” if they serve only as an energy source to extend adult longevity [24]. Therefore, results of this study confirm that *A. gossypii* was identified as an essential prey, while bee pollen and rice moth eggs were classified as alternative prey for *C. inaequalis*.

5. Conclusions

Bee pollen and rice moth eggs serve as alternative prey for adult *C. inaequalis*, providing temporary sustenance; however, exclusive consumption of these diets results in unsuccessful fecundity. 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 and higher death rate, compared to those fed solely on *A. gossypii*. Furthermore, the 1st instar larvae of *C. inaequalis* showed no acceptance of bee pollen or rice moth eggs. This presents an intriguing opportunity for further research on the potential effects of offering bee pollen or rice moth eggs to other larval instars of *C. inaequalis* and possibility of applying these diets as artificial food for *C. inaequalis* mass rearing.

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Data Availability Statement: All datasets supporting the conclusion of this article are include in the article. Data will not be shared in any other source.

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Conflicts of Interest: The authors declare no conflicts of interest.

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