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PREFERENCE OF THE METALLIC BLUE LADYBEETLE *CURINUS COERULEUS* MULSANT FOR DIFFERENT NYMPHAL INSTARS OF *DIAPHORINA CITRI* KUWAYAMA (HOMOPTERA: PSYLLIDAE)

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Abstrak: Kecenderungan kumbang biru metalik, *Curinus coeruleus* Mulsant terhadap kutu limau Asia, *Diaphorina citri* Kuwayama telah dinilai di insektari. Objektif kajian ini adalah menentukan sama ada terdapat perbezaan kecenderungan *C. coeruleus* pada peringkat yang berbeza terhadap perbezaan instar *D. citri*. Larva kumbang pada instar pertama dan ke-2 menunjukkan kecenderungan terhadap mangsa bersaiz kecil, sedangkan larva pada instar ke-3, ke-4 dan kumbang dewasa lebih cenderung terhadap nimfa *D. citri* pada instar ke-4 dan ke-5. Nilai kecenderungan paling tinggi direkodkan oleh setiap peringkat pemangsa, larva instar pertama ke instar ke-4 dan dewasa, ialah 0.49, 0.29, 0.33, 0.33 dan 0.34 masing-masing menurut peringkat nimfa pertama, ke-2, ke-4, ke-4 dan ke-4. Kebarangkalian tangkapan oleh setiap peringkat pemangsa menunjukkan perbezaan yang signifikan dalam kalangan peringkat instar mangsa. Larva instar pertama dan ke-2 menangkap dengan perbezaan yang signifikan peringkat nimfa pertama dan ke-2 berbanding nimfa peringkat lain. Sebaliknya, larva pemangsa instar ke-3, ke-4 dan dewasa menangkap dengan perbezaan yang signifikan nimfa ke-4 dan ke-5. Justeru, keputusan kajian mencadangkan bahawa pemangsa *C. coeruleus* berkecenderungan menangkap mangsa *D. citri* yang bersaiz sama dengannya atau yang lebih kecil. Kecenderungan ini berlaku kerana ia berkait dengan kebarangkalian untuk berjaya menangkap mangsa.

Abstract: The preference of the metallic blue ladybeetle, *Curinus coeruleus* Mulsant for Asian citrus psyllid, *Diaphorina citri* Kuwayama was investigated in the insectary. The aim was to determine whether there was any preference by *C. coeruleus* at different stages for different instars of the psyllid *D. citri*. The 1st and 2nd instar larvae exhibited preference for smaller-sized prey, while the 3rd and 4th instar larvae and adult mostly preferred the 4th and 5th instar nymphs. The highest preference value recorded for each stage of the predator, 1st to 4th instar larvae and adult, were 0.49, 0.29, 0.33, 0.33 and 0.34, respectively on the 1st, 2nd, 4th, 4th and 4th instar nymphs. The probability of capture indicated significant differences among prey instars captured by each stage of predator. The 1st and 2nd instar larvae captured significantly more 1st and 2nd instar nymphs than the other instar nymphs. In contrast, 3rd and 4th instar larvae and adult predator captured significantly more 4th and 5th instar nymphs. Hence, the results suggest that *C. coeruleus* exhibited body size preference for *D. citri* nymphs comparable to or slightly smaller than predator's size (stages). This preference was due to the probability of success in capturing the prey.

Keywords: Preference, *Curinus coeruleus*, *Diaphorina citri*

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INTRODUCTION

The Asian citrus psyllid *Diaphorina citri* Kuwayama is known as an efficient vector of the transmittable endocellular, phloem-restricted bacterium, *Liberobacter asiaticum*, the causal organism of citrus greening disease. This disease is a destructive malady seriously affecting most commercial important citrus cultivars in terms of causing reduced production and death of trees. Ko (1996) suggested that the citrus greening disease would become the worst ever threat to the citrus industry in Malaysia. Fifty-two per cent of *Citrus suhuiensis* var. 'Limau Madu' grown in Peninsular Malaysia indicated positive symptom of the greening disease based on polymerase chain reaction (PCR) test (Anonymous 2001).

Under natural conditions, both nymph and adult psyllids are easily found on neglected citrus and jasmine orange plants. The population of this insect is entirely dependent upon the availability and abundance of new plant shoots. Its population is also adversely affected by various species of natural enemies. The citrus psyllid nymphs are especially preyed upon by the ladybird beetles *Cheilomenes sexmaculata* (Waterhouse 1998; Chien & Chu 1996), *C. quadriplagiata* and *Chilocorus nigritus* (Aubert 1989), and the metallic blue *Curinus coeruleus* Mulsant (Anonymous 1999; Michaud *et al*. 2002). The latter has been reported to be able to reduce the population of citrus psyllids in Indonesia (Anonymous 1999) and in Florida (Michaud *et al*. 2002); however, less is known of its predator-prey interaction with the citrus psyllids.

This paper reports the results of studies intended to understand how the preference of *C. coeruleus* at different stages when presented to the mixtures of different instars of *D. citri*, as in nature, the citrus psyllid population comprises a mixture of different instars.

MATERIALS AND METHODS

Effects of Prey Developmental Stages on the Preference of Predator

Both *D. citri* nymphs and the predator *C. coeruleus* were obtained from stock cultures. Mass culture of *D. citri* was maintained on the orange jasmine plants *Muraya paniculata* (L.) in the glass house condition of 28–34ºC and 60–75% RH, while *C. coeruleus* was reared on an excessive provision of psyllid nymphs of *D. citri* and *Heteropsylla cubana* (psyllid infesting *Leucaena leucocephala*) in the insectary. *D. citri* has five nymphal instars and all these instars were used in the experiment. Predators used consisted of four larval instars and young adult females, which were on the average larger than the males (Dixon 2000; Mizell & Nebeker 1982).

The study of preference for prey nymphs was conducted by placing the predators individually in an inverted 12 cm glass petri dish lined with a moistened filter paper and provided with the psyllid nymphs on a fresh *Murraya* leaflet as the arena following the method modified from Maelzer (1978) and Hussein (1991). Five treatments consisting of different combinations of *D. citri* instars were each provisioned to five predator stages on each of the days of the larval development and on each of the days 1–4 of adult (Table 1). Every day the treatment was randomized amongst individual predator. Each treatment was replicated five times. Preference of the predator to different prey instars was measured by graphical analysis which displayed the difference between the actual and expected proportion of *D. citri* nymphs attacked by the predator. The actual proportion of nymphal instars attacked in one day was estimated by totalling the numbers of that instar attacked that day by each of the five individual predator in the five treatments and divided by the total number of prey instars actually attacked. Meanwhile the expected proportion of prey instar attacked was computed by totalling the number of that instar presented to each of the five individual predator in the five treatments and divided by the total number of all prey instars initially presented.

Table 1: Treatment combinations of nymphal instars of *D. citri* fed to various developmental stages of *C. coeruleus.*

Treatment combination	No. of instars of *D. citri* offered									
	1st, 2nd instar larvae					3rd, 4th instar and adult predator				
	I	II	III	IV	V	I	II	III	IV	V
A	30	20				20	20	20	20	20
B	30		20			20	30	20	15	15
C	30			20		30	20	15	20	15
D	30				20	30	15	20	15	20
E	10	10	10	10	10	15	20	20	20	15
Total	**130**	**30**	**30**	**30**	**30**	**115**	**105**	**95**	**90**	**85**

The preference of any predator instar over different prey instars was quantitatively estimated by Manly's preference index as proposed by Chesson (1978).

$$\alpha_i = \frac{r_i / n_i}{\sum_{j=1}^{m} r_j / n_j}, i = 1, \ldots, m$$

where α_i = preference scaled so that the values of α in a given experimental treatment sum to 1

r_i = the proportion of prey instar *i* attacked by predator

n_i = the proportion of prey instar *i* presented to predator

Effects of Prey Developmental Stages on the Probability of Capture by the Predator

Probability of capture, which is the ratio of percentage of instar attacked and percentage of total prey, was estimated from data obtained from the experiment. The percentage of instar attacked was estimated by totalling the numbers of that instar attacked and divided by the total number of prey presented. The levels of probability of capture by each predator stages over each instar of *D. citri* were subjected to the one-way analysis of variance using MSTAT software (MSTAT 1986) and the means were separated by least significant difference (LDS) at 5% level of probability. Since data analysed were percentages, ranging from 0–100% arcsine transformation was applied to homogenize the variance prior to the analysis of variance (Gomez & Gomez 1984).

RESULTS AND DISCUSSION

Each stage of the predator *C. cueroleus* demonstrated an obvious preference for certain nymphal instars with low proportions of prey (Fig. 1 & 2). The younger predators, i.e. 1st and 2nd instars, somewhat appeared to prefer younger nymphs of *D. citri* as indicated by the positive percentage difference between the actual and the expected proportions of prey attacked. However during their development, the preference of these early instar larvae tended to shift gradually from small-sized *D. citri* nymphs to larger-sized. This was clearly evidenced by the gradual reduction in the positive preference of the 1st and the 2nd instar nymphs and also by the progressively smaller negative preference of the larger nymphal instars. As the larval predator entered the 3rd instar (Fig. 1) and continued through the adult stage (Fig. 2), the preference over the prey stages shifted to the larger nymphs, especially 4th and 5th instar nymphs. It was apparent that the large predators had large negative preferences over the 1st and 2nd instar nymphs.

The preference indices of *C. coeruleus* for the various nymphal instars of *D. citri* are presented in Table 2. Preference index for the 1st instar larvae was highest when offered 1st and 2nd instar nymphs, while the preference for the 3rd and 4th was negligible, and the 5th instar nymph was not at all attacked by the 1st instar larvae. The preference of the 1st instar larvae was 1.32:1 in favour of the 1st instar over the 2nd instar nymphs. Similarly, the preference of the 2nd instar larvae was 1.21:1 for the 2nd instar over the 1st instar nymphs. For the larger larvae and the adult predators, preference indices were higher for large nymphs rather than the small ones, indicating that the large predators preferred larger nymphs. In general, among the nymphal instars presented to large predators, the 4th instar was the most preferred, followed by the 5th and the 3rd instars.

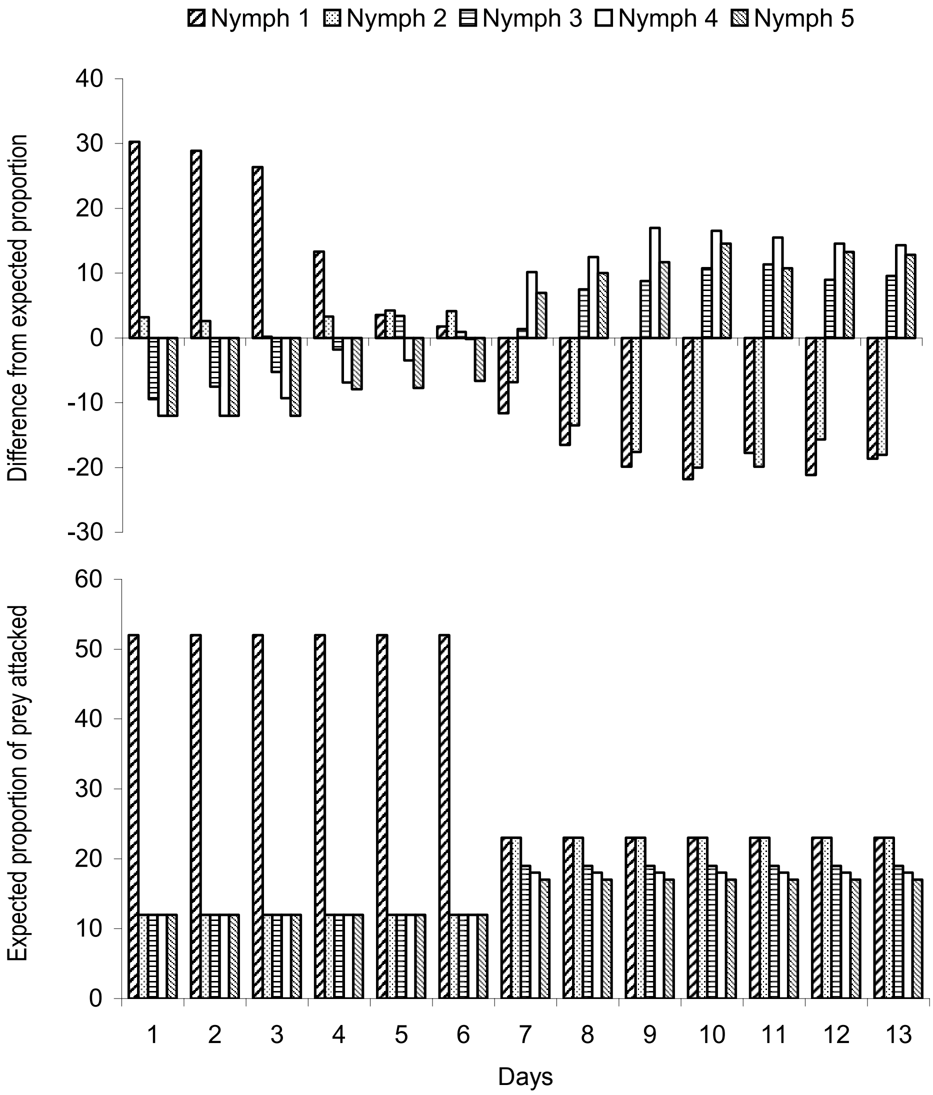

Figure 1: The proportion of different nymphal instars of *D. citri* presented, and the difference between proportion of *D. citri* nymph presented and attacked by predator larvae.

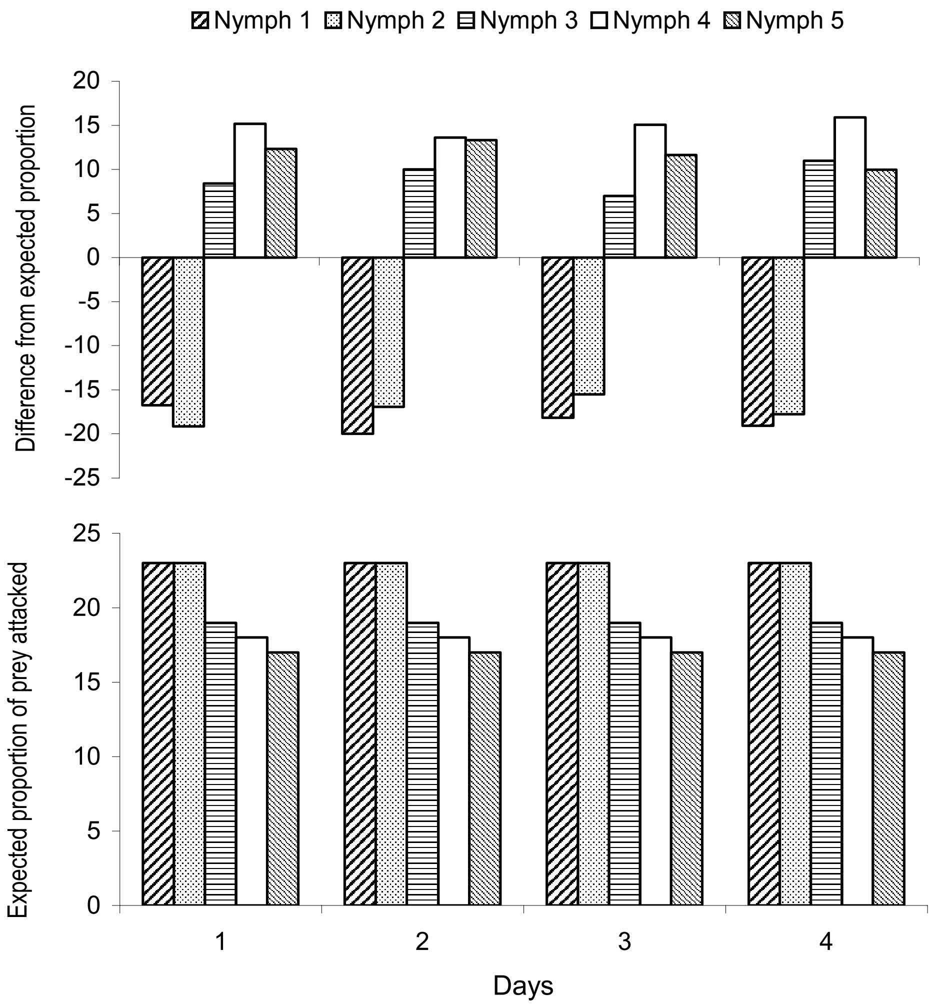

Figure 2: Proportion of different nymphal instars of *D. citri* presented, and the difference between proportion of *D. citri* nymph presented and attacked by adult predator.

Table 2: Manly's preference index for various developmental stages of *C. coeruleus* presented with different nymphal instars of *D. citri.*

Nymphal instars of *D. citri*	Mean Manly's preference index for *C. coeruleus*				
	Larval instars				Adult
	1st	2nd	3rd	4th	
I	0.49	0.24	0.04	0.03	0.04
II	0.37	0.29	0.07	0.04	0.04
III	0.12	0.23	0.25	0.28	0.27
IV	0.02	0.15	0.33	0.33	0.34
V	0	0.08	0.31	0.32	0.31

Preference or selection of prey is influenced by two important factors, i.e. prey acceptance and prey suitability (Lucas *et al*. 1997). Prey acceptance is determined by the capture success of the predator and by its acceptance or rejection of the prey after 'tasting'. The capture success of the predator as a measure of the probability of capture is calculable from the ratio of percentage of instar attacked and percentage of total prey (Chesson 1978; Maelzer 1978). In this study, the preference expressed by the probability of capture by each stage of the predator for different nymphal instars was significantly different (Table 3). For the 1st and 2nd instar *C. coerulius*, the highest probability of capture was observed with the 1st and 2nd instar nymphs of *D. citri*; while for the 3rd and 4th instar larvae and the adult predators, the highest probability of capture was observed with the 4th instar nymphs. The data also revealed clearly the similarity of the ranking order between preference indices in Table 2 and probability of capture in Table 3, indicating close relationships between the two parameters.

Table 3: Probability of capture of different nymphal instars of *D. citri* by *C. coeruleus.*

Nymphal instars of *D. citri*	Probability of capture by *C. coeruleus* (%)				
	Larval instars				Adult
	1st	2nd	3rd	4th	
I	45.00 ± 3.1 a	42.63 ± 3.7 a	16.35 ± 7.1 b	14.08 ± 3.2 c	17.05 ± 2.3 c
II	37.86 ± 4.1 a	49.24 ± 4.4 a	20.94 ± 7.3 b	16.80 ± 4.5 b	19.51 ± 3.1 c
III	20.17 ± 4.7 b	41.75 ± 8.1 a	46.37 ± 7.2 a	49.56 ± 4.8 a	54.58 ± 3.8 b
IV	5.33 ± 3.2 c	32.22 ± 7.1 b	57.35 ± 9.6 a	56.52 ± 6.6 a	64.96 ± 3.1 a
V	0.50 ± 0 c	23.21 ± 1.6 c	54.76 ± 6.9 a	54.20 ± 6.7 a	60.67 ± 3.2 a

Means ± standard error within columns followed by the same letter are not significantly different at 5% level of LSD.

Results from this study suggest that body size of both the predator and the prey appeared to be an important factor in prey preference or capture. The smaller predator exemplified its preference just for the smaller preys, while the larger larval instars and the adult predators preferred the larger preys. Mizell and Nebeker (1982) mentioned that size of the prey and ease of handling contributed to the preference, as such much larger or smaller, in comparison with the size of suitable prey, was rejected. The large preys are easier to be seen and encountered, however, the fact is that only the bigger larvae (3^{rd} and 4^{th} instar) and the adult had high probability of capture and showed positive preference. This preference by large larvae and adult predator is significant in biological control of the Asian citrus psyllid, *D. citri* since the 4^{th} and 5^{th} instar nymphs are able to transmit the citrus greening disease and transfer their inoculative capacity to their freshly emerged adults (Aubert 1990; Halbert 1999).

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