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Prey preference of an aphidophagous ladybird, *Coccinella transversalis* (Coccinellidae: Coleoptera) on two aphid species

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Abstract

Prey Preference of adult male and female, *Coccinella transversalis* (Fabricius) was studied when fed on aphids, *Aphis craccivora* (Koch) and *Aphis gossypii* (Glover), which sequester toxic allelochemicals from their host plants. Adult males and females of *C. transversalis* prefer to consume *A. gossypii* over *A. craccivora* in most mixed diet combinations (cafeteria setup). This was strongly supported by significantly high values of β and C prey preference indices. However, when provided with *A. craccivora* and *A. gossypii* separately, we found a similar reluctant behaviour in the adults. The adults showed no significant difference in aphid consumption, regardless of the species of aphid and the sex of the adult ladybird. Therefore, we conclude that host plant allelochemicals/ toxicants have a direct effect on the prey preference of ladybirds. Mixing two toxic similar diets can make one diet more suitable than the other.

Keywords: *Coccinella transversalis*, *Aphis craccivora*, *Aphis gossypii*, *Ranunculus sceleratus*, diet

Introduction

Food is a crucial fundamental factor determining the ladybirds' growth, development and reproduction. Predaceous ladybirds can play a significant role as biocontrol agents (Michaud, 2012) [12], however, very few of them are considered potential biocontrol agents (Evans *et al.*, 2011; Roy *et al.*, 2016) [3, 19]. Both quality and quantity of prey species are key components that influence the life history traits of insects at individual and species levels (Blackenhorn *et al.*, 2000) [2]. Among the foods of predaceous ladybirds, the essential foods support both development and reproduction, while the other foods including factitious foods, which they consume during scarcity or absence of the essential food, can only support the survival of the individual.

Aphidophagous ladybirds, primarily, prefer feeding on aphids, however, aphid suitability may depend on various factors (Rana *et al.*, 2002; Soares *et al.*, 2004) [18, 22]. Diet suitability to aphidophagous ladybirds seems unpredictable, as the same aphid can be both toxic and nutritious under different circumstances (Guroo *et al.*, 2017) [6]. Toxic aphids can become nutritious if ladybirds may continuously feed them for several generations (Rana *et al.*, 2002) [18]. The toxicity of aphids directly depends on the host plant's toxic allelochemicals, which they sequester (Pratt *et al.*, 2008) [17] as a means of defence against predators and may reduce the predators' growth rates (Noriyuki *et al.*, 2012) [14]. Soares *et al.* (2004) [22] demonstrated the process of dietary self-selection in a ladybird, *Harmonia axyridis* (Pallas), whose voracity increased with an increase in the proportion of *Myzus persicae* (Sulzer) in the population, when provided with mixed populations of aphids, *Aphis fabae* Scopoli and *M. persicae*. The males constantly preferred *M. persicae*, while females didn't.

Hodek and Evans (2012) [8] consider the black bean aphid, *Aphis craccivora* (Koch) to be toxic due to allelochemicals, *viz.* amines canavanine and ethanolamine (Obatake and Suzuki 1985) [15] that it sequesters from bean plants (Hokusima and Kamei 1970). The buttercup, *Ranunculus sceleratus* L. is a toxic plant with global distribution containing toxic allelochemicals that aphids, particularly *A. craccivora*, may easily sequester (Gupta and Singh 1983; Aslam *et al.* 2012) [5, 1]. Aphid, *Aphis gossypii* (Glover), is a major pest of bottle gourd (*Lagenaria vulgaris*) and is also widely distributed due to its broad host plant range.

Coccinella transversalis (Fabricius) is an aphidophagous ladybird of the Oriental region with a wide prey-range and can be found preying upon the colonies of aphids infested on local agricultural and horticultural crop plants. Quality food is directly associated with an increase in its body weight (Pervez and Sharma, 2021) [16]. Since plant allelochemicals have a major role in the preference and suitability of prey, we hypothesize that *A. craccivora* reared on the toxic *R. sceleratus* will be less preferred when mixed with different proportions of *A. gossypii*. So, the present study was undertaken to observe the prey preference of *C. transversalis* for two aphid species, i.e. *A. gossypii* and *A. craccivora* (reared on toxic plants).

Materials and Methods

Stock Maintenance

Adults of *C. transversalis* were collected from the agricultural fields of Kashipur, Uttarakhand, North India (30.2937°N, 79.5603°E) and were brought to the laboratory. They were allowed to mate in the Petri dishes (2.0 cm × 9.0 cm) containing an *ad libitum* supply of the aphids, *A. craccivora* and *A. gossypii* infesting on the pieces of leaves/twigs of *R. sceleratus* and *L. vulgaris*, respectively (n = 10), and were kept in an Environmental Test Chamber (REMI Instruments, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L: 12D. Mating pairs were observed daily and any eggs laid were transferred to a new Petri dish (size as above). Eggs when hatched, neonates were transferred to the muslin-covered beakers and were provided with an *ad libitum* diet until they completed development. The emerging F₁ adults were sexed, isolated and raised on their parental diets.

Prey preference in a cafeteria setup

To study the prey preference, aphids, *A. craccivora* (*Ac*) and *A. gossypii* (*Ag*) were offered to the adult male and female of *C. transversalis* in three different ratios of *Ac*: *Ag* (50: 100, 75: 75 and 100: 50 respectively).

A 12-hour starved 10-day-old adult male *C. transversalis* was placed in a beaker containing plant twigs of the respective host plants (as mentioned above) of the two aphid species viz. *Ac* and *Ag* in the ratio 50:100 respectively. The beaker was covered with a muslin cloth fastened with a rubber band and kept in the Environmental Test Chamber (REMI Instruments, India) maintained at 27 ± 1 °C, 65 ± 5% R.H and 12L: 12D. Similar treatments were done with the other two mixtures of aphids with varied ratios (*Ac*: *Ag* = 75: 75 and *Ac*: *Ag* = 100: 50). After 24 hours, the beaker was taken out and the number of unconsumed aphids were counted to quantify the number of aphids consumed. The experiment was replicated ten times (n = 10). The experiment was repeated using adult female *C. transversalis* as the predator. We calculated Manly's preference index (Manly 1972) [11] for each treatment using the formula, $\beta = \log (NA / rA) / [\log (NA / rA) + \log (NB / rB)]$, where NA and NB are the number of prey A and prey B offered to the ladybird and rA and rB are the numbers of unconsumed prey. If β is close to 1, the predator prefers prey A and if close to 0, prey B. An index value close to 0.5 indicates no preference. We tested the β obtained for each treatment for significant difference from a value of (0.5) using a one sample t-test and statistical software, SAS Version 9.0. Prey preference was also analyzed using the C index ($C = (EA \times NB) / (EB \times NA)$) (Sherratt and Harvey 1993), where EA and EB are the number of prey A and prey B consumed. A 'C' value between 0 and 1 indicates a preference for prey B and a value more than 1 indicates preference for prey A. We

subjected the C-index for each treatment to a one sample t-test to determine whether it was significantly different from a value of 1.0 using SAS 9.0. We subjected data on the number of prey consumed to Wilcoxon's matched-pairs signed rank test and the proportion of each prey consumed in a cafeteria setup to a two sample t-test in SAS Version 9.0.

Response to monotypic diet

To determine the feeding propensity, adult male and female of *C. transversalis* were each provided with a monotypic diet of *Ac* or *Ag*. An adult male was kept in a glass beaker containing 50 third instars of *Ac* infested twig of the respective host plant (as above). The beaker was covered with a muslin cloth fastened with a rubber band and kept in the Environmental Test Chamber (REMI Instruments, India) at 27 ± 1 °C, 65 ± 5% R.H and 12L: 12D. After 24h, the beakers were taken out and the number of live aphids counted to quantify the number of aphids consumed (n = 10). The experiment was repeated using adult female *C. transversalis* as the predator. The data was tested on prey consumption for normality using the Kolmogorov–Smirnov test and homogeneity of variance using Bartlett's test in statistical software, SAS Version 9.0. The data on the consumption of aphids, when provided with a monotypic diet, were subjected to a two-sample t-test using SAS 9.0 and the means were compared. We subjected the data on the number of the two species of aphids consumed by the adult male and female *C. transversalis* to a two-way ANOVA using 'aphid species' and 'sex' as independent variables and 'prey consumed' as dependent variable in SAS 9.0.

Results

Prey preference in a cafeteria setup

Adult male of *C. transversalis* preferred to consume aphid, *A. gossypii* over *A. craccivora* at all three ratios of the mixed diet tested, i.e. at 50 :100 (*Ac*: *Ag*) ratio ($Z_{(1, 18)} = -2.701$; $P = 0.0069$), 75:75 (*Ac*: *Ag*) ratio ($Z_{(1, 18)} = -2.803$; $P = 0.005$) and 100:50 (*Ac*: *Ag*) ratio ($Z_{(1, 18)} = -2.701$; $P = 0.0069$) [Fig. (1.a)]. However, adult female ladybirds only preferred *A. gossypii* over *A. craccivora* when an equal proportion of the aphids were provided 75: 75 (*Ac*: *Ag*) ($Z_{(1, 18)} = -2.701$; $P = 0.0069$) and at the 100: 50(*Ac*: *Ag*) ratio ($Z_{(1, 18)} = -2.599$; $P = 0.009$) and at the 100 *Ac*: 50 *Ag* ratio ($Z_{(1, 18)} = -2.599$; $P = 0.009$) [Fig. (1.b)]. The proportion of prey consumed at the 50: 100 (*Ac*: *Ag*) ratio by female *C. transversalis* was not statistically significant ($Z_{(1, 18)} = -2.599$; $P = 0.009$; Fig. 1b). Two-way ANOVA revealed a significant main effect of 'aphid' species on the proportion of prey consumed ($F = 44.20$; $p < 0.0001$; d.f. = 1). The main effect 'sex' was not found to be statistically significant ($F = 44.20$; $p < 0.0001$; d.f. = 1). The interaction between 'sex' and 'aphid' did not differ significantly ($F = 2.64$; $P = 0.107$; d.f. = 1). Both β and C indices had significantly negative t-values in all the combinations except for adult females provided with the aphid proportion 50 :100 (*Ac*: *Ag*) (Table. 1).

Response to a monotypic diet

Both adult male ($t = -0.22$; $P = 0.83$; d. f. = 1, 9) and female ($t = 0.92$; $P = 0.383$; d. f. = 1, 9) of *C. transversalis* equally consumed the aphids, *A. craccivora* and *A. gossypii* raised on toxic hosts (Fig. 2). Hence the prey consumption was not found to be statistically significant. The two-way ANOVA revealed that the main effects 'aphid species' ($F = 0.11$; $P = 0.745$; d. f. = 1) and 'sex' ($F = 0.18$; $P = 0.713$; d.f. = 1), along with the interaction 'aphid species' x 'sex' ($F = 0.43$; $P = 0.516$; d.f. = 1, 39) were not statistically significant.

Table 1: Mean values of the β and C indices of *C. transversalis* with different proportions of the aphids *A. craccivora* and *A. gossypii*.

Life stage	Prey ratio	β index	t-value	C index	t-value
Adult Male	50:100	0.44±0.02	t = -3.57; p<0.001	0.84±0.03	t = -3.87; p<0.001
	75:75	0.37±0.02	t = -6.98; p<0.0001	0.67±0.03	t = -8.14; p<0.0001
	100:50	0.37±0.03	t = -5.54; p<0.0001	0.69±0.04	t = -5.92; p<0.0001
Adult Female	50:100	0.46±0.03	t = -1.06; p<0.001	0.95±0.06	t = -0.40; p = 0.7
	75:75	0.42±0.02	t = -4.58 p<0.0001	0.80±0.04	t = -4.23; p<0.001
	100:50	0.42±0.02	t = -4.14; p<0.001	0.78±0.04	t = -4.07; p<0.001

*The predator prefers *Aphis craccivora* if β is close to 1 and *Aphis gossypii* if β is close to 0; β close to 0.5 indicates no preference. C index of more than 1 indicates a preference for *Aphis craccivora*, while C index between 0 and 1 indicates a preference for *Aphis gossypii*.

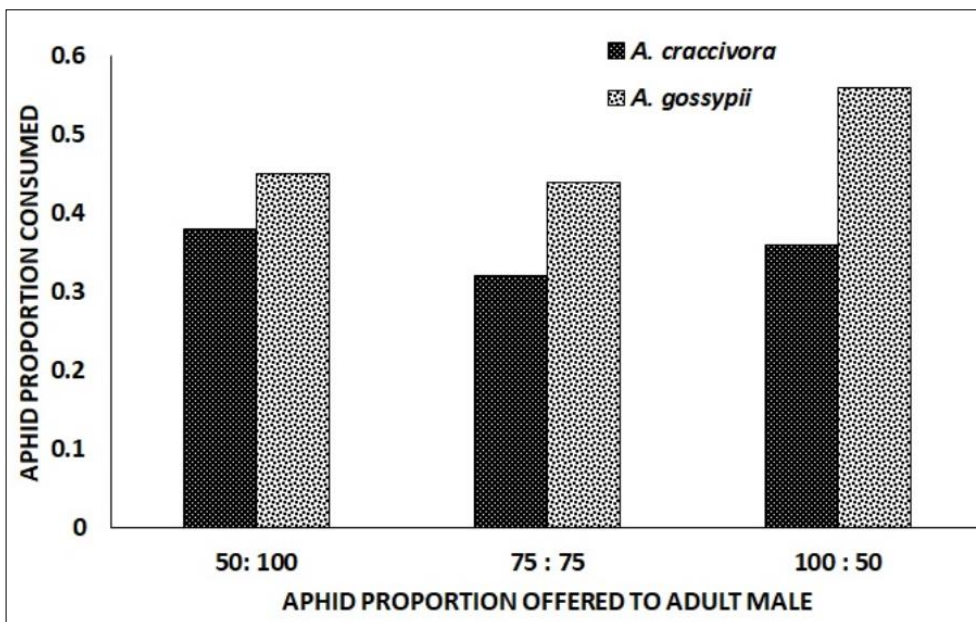


Fig 1(a): Proportion of aphids (*A. craccivora*: *A. gossypii*) consumed by adult male of *C. transversalis*.

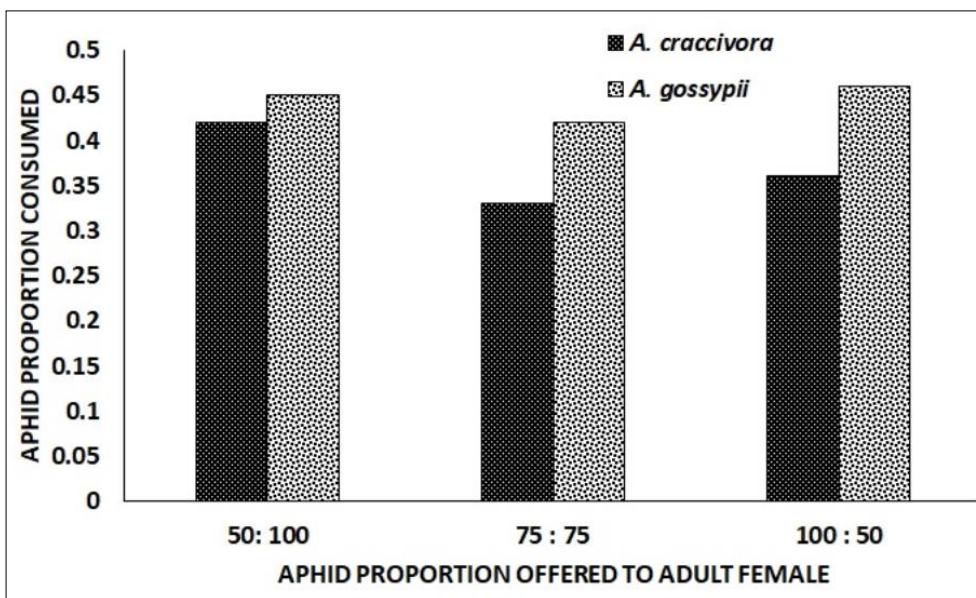


Fig 1(b): Proportion of aphids (*A. craccivora*: *A. gossypii*) consumed by adult female of *C. transversalis*.

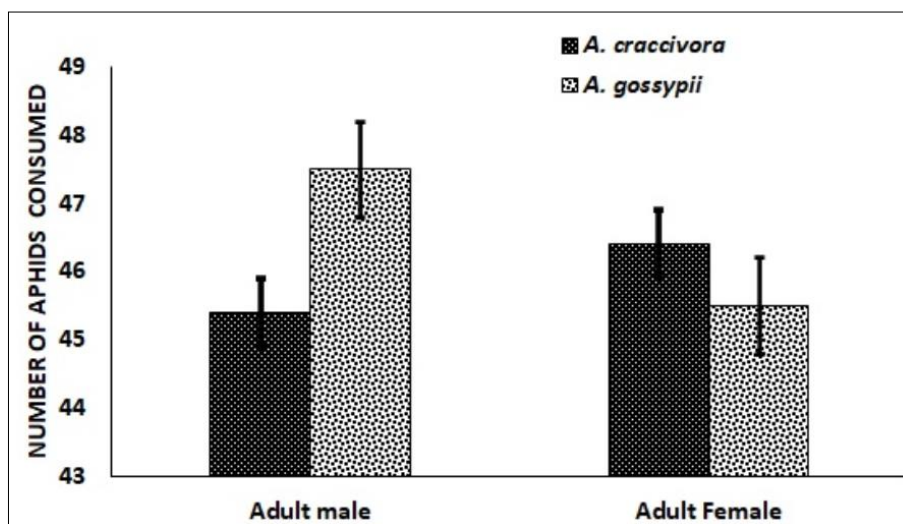


Fig 2: Prey consumption by adult male and female *C. transversalis* when fed on monotypic aphid diet.

Discussion

In all mixed diet combinations, both adult male and female *C. transversalis* consumed more *A. gossypii* than *A. craccivora*. This supports our hypothesis that *A. craccivora* reared on the toxic *R. sceleratus* will be less preferred when mixed with *A. gossypii*. It is also evident from the preference (β and C) indices that *A. gossypii* is preferred over the toxic *A. craccivora*. Therefore, our findings agree with that of Guroo *et al.* (2017) [6], who reported similar preference indices for the larvae and adults of *Coccinella septempunctata* L. for *Lipaphis erysimi* (Kalt.) when mixed with the toxic prey, *Brevicoryne brassicae* L. It has become clear from the present findings that plant toxicants and allelochemicals have a direct effect on the quality of aphids in terms of their toothsome for predators. *Ranunculus* sp. has secondary metabolites, like glycosides, phenolic compounds, steroids, di and tri terpenes, coumarins and flavanoids, which when sequestered by herbivores may harm predators (Hachelaf *et al.*, 2013) [23]. Non-preference for *A. craccivora* indicates that allelochemicals from *Ranunculus* sp. *i.e.* its host plant are more toxic and have a greater effect on the quality of *A. craccivora*. The seven-spotted ladybird, *C. septempunctata* eats twice as many of the toxic aphid, *Aphis sambuci* L. than of the nutritious aphids, *viz.* *Acyrtosiphon pisum* (Harris) and *Aphis philadelphia* (Nedved and Salvucci 2008) [13]. The two-spotted ladybird, *Adalia bipunctata* (L.) consumes more *A. craccivora* than the more suitable aphid, *Acyrtosiphon pisum* (Harris) (Ferrer *et al.* 2008) [4]. Similarly, Senkeřiková and Nedvěď (2013) [20] reported that the Harlequin ladybird, *Harmonia axyridis* (Pallas) consumes a greater number of the toxic aphid, *A. sambuci* than the suitable aphid, *Aphis fabae cirsiacanthoidis* (syn. *A. philadelphia*). Soares *et al.* (2004) [22] report that a mixed aphid diet is better than a monotypic aphid diet in certain situations (Soares *et al.*, 2004) [22]. Similar behaviour of both adult male and female, *C. transversalis* was found when provided with monotypic diets. The main effects of 'aphid species' and 'sex' were not statistically significant, which reveals similarity in terms of biochemical contents of the two aphids, and the response of the two sexes of adults towards these aphids. This indicates that both prey were consumed equally when provided separately, and the significant preference for *A. gossypii* in the cafeteria experiment reveals that prey preference was skewed towards this prey. This further affirms our theory that possible vital nutrients missing in *A. gossypii* might have been

supplemented by eating *A. craccivora*. We conclude that: (i) host plant allelochemicals have a direct effect on the prey preference of ladybirds, (ii) a change in host plant can result in a most preferred prey becoming less preferred, and (iii) mixing two toxic similar diets can make one diet more suitable than the other.

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