



## Intraguild predation of inferior larval instars of two ladybirds *Menochilus sexmaculatus* (Fabricius) and *Propylea dissecta* (Mulsant) (Coleoptera, Coccinellidae)

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**ABSTRACT:** Ladybird beetles are predatory insects, which consume several insect pests and have immense biocontrol potential, particularly against aphids. In prey scarcity, they resort to intraguild predation (IGP) by consuming immature stages of other heterospecific ladybirds. A laboratory experiment was performed to quantify the incidence of IGP of first and second larval instars by older instars and adults of two co-occurring ladybird species, *Propylea dissecta* and *Menochilus sexmaculatus*. IGP of first and second larval instars increased significantly with increase in the larval stages followed by adult males and females. Predatory stages of *M. sexmaculatus* were more potential intraguild predators than those of *P. dissecta*. Among adults, the female consumed a greater number of early and weaker heterospecific instars. The presence of dorsal spines and hair on the larva of *M. sexmaculatus* provided aposematism that helps in defense against superior heterospecific larvae thereby enabling its successful establishment and distribution. *M. sexmaculatus* could act as an intraguild predator in the fields and may contribute in declining the population density of *P. dissecta* during aphid-prey scarcity.

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**KEY WORDS:** Population density, heterospecific instars, aposematism, aphid-prey scarcity

### INTRODUCTION

The majority of predaceous ladybirds (Coleoptera, Coccinellidae) are economically important for their biocontrol potential against several phytophagous insect pests, viz. aphids, coccids, diaspidids and aleyrodids (Hodek *et al.*, 2012). Older larval instars used to prey upon eggs and the early instars of heterospecific ladybirds during aphid scarcity (Pervez *et al.*, 2021). Such interactions are referred to as intraguild predation (IGP), where two predators compete for a common prey resource,

which are frequent in ladybirds (Polis *et al.*, 1989; Khan and Yoldas, 2018). The dominant predator (IG predator) attacks and consumes the inferior one (IG prey) sharing common prey resource (extraguild prey) (Lucas, 2012). The terms, 'superior' and 'inferior' are used when one species (Reitz and Trumble, 2002; Putra *et al.*, 2009), competitor (Inouye, 2005), larval stage (Pervez *et al.*, 2021) or aphid-prey (Bilu and Coll, 2009) dominates the other one. IGP may disrupt biocontrol programmes (Mansfield, 2019) and can endanger biodiversity (Smith and Gardiner, 2013) by attacking

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inferior and native coccinellid species of different agroecosystems.

Exotic invading ladybirds may dominate the agroecosystems by competing for a shared prey, declining native coccinellid populations by IGP and overlapping niche (van Lenteren *et al.*, 2006). IGP in ladybirds is generally bidirectional and asymmetrical (Rondoni *et al.*, 2014; Rocca *et al.*, 2017). Usually, adults and higher coccinellid instars attack immobile and poorly-defended vulnerable life stages like eggs and lower instars (Lucas, 2005; Hemptinne *et al.*, 2011). Eggs and inferior larvae of conspecifics (Pervez *et al.*, 2006; Roy *et al.*, 2007) and heterospecifics (de Clercq *et al.*, 2005; Michaud and Jyoti, 2008) are even more nutritious than aphids to ladybirds in certain cases.

*Propylea dissecta* (Mulsant) and *Menochilus sexmaculatus* (Fabricius) are oriental ladybirds, which abundantly occur in the agricultural fields preying on aphids (Omkar and Pervez, 2004). The coccinellid *P. dissecta* can consume aphids raised on both nutritious and toxic hosts, which reveals its sustainability in the aphid available agro-ecosystems (Pervez and Omkar, 2011) and possesses immense biocontrol potential (Bhoopathi *et al.*, 2020). It co-occurs with many coccinellid species, including *M. sexmaculatus*. Similarly, *M. sexmaculatus* feeds on a vast range of aphid-prey (Pervez and Chandra, 2018), however little is known on its intraguild interactions. Singh *et al.* (2020) found that it may resort to oophagy with deteriorating fitness during aphid scarcity. The two ladybird species can easily be mass-reared for the augmentative release as aphid biocontrol agents. Singh *et al.* (2016) emphasized on the slow and fast developing individuals in these two ladybird species in a common habitat. Despite their co-occurrence, *M. sexmaculatus* seems to have a better compensatory ability than *P. dissecta* to overcome the stress of fluctuating prey-resource (Chaudhary *et al.*, 2016). Prescott and Andow (2019) suggested ineffective avoidance mechanisms to be the possible reason for the co-occurrence of IG predators. However, the question on the possible impact of these ladybirds on each other's abundance during prey-scarcity is still unaddressed. An

investigation was undertaken to assess the potential intraguild predator and the intraguild prey when the two ladybird species co-occur in the absence of natural aphid prey and the stage-specific larval interaction during intraguild combat between the two ladybird species.

## MATERIALS AND METHODS

### Stock Culture

Adults of *M. sexmaculatus* and *P. dissecta* were collected from the agricultural fields near the suburbs of Kashipur, Uttarakhand (29°21'04"N; 78°9'19"E), and brought to the laboratory. These adults were paired in the Petri dishes (9.0 × 2.0 cm) consisting of *ad libitum* supply of aphids, *Aphis craccivora*, infested on the bean (*Dolichos lablab* L.) twigs along with moist filter paper under constant conditions (27 ± 2°C; 65 ± 5% RH; 14L: 10D) in an Environmental Test Chamber (REMI, Remi Instruments). The adults mated and laid F<sub>1</sub> eggs, which were reared from egg-hatch to adult emergence in the above mentioned abiotic and biotic conditions (using the above prey). The emerging F<sub>1</sub> adults were again sexed, isolated, paired, and allowed to mate. The F<sub>2</sub> eggs laid by the F<sub>1</sub> adult females after mating, were collected and isolated. These F<sub>2</sub> eggs were used to develop the larval instars needed for the experimental design.

### Experimental Design

#### *Propylea dissecta* as an intraguild predator

Ten first-instars (10 h old) of *M. sexmaculatus* were released as intraguild prey in a Petri dish). Thereafter, one-day-old second instar of *P. dissecta* was released as an intraguild predator in the same Petri dish and allowed to consume the first instars. After 12 h, the second-instar predator was removed from the Petri dish and the numbers of remaining first instars were counted to determine the number of first instars consumed. The experiment was replicated ten times (n = 10). The same experiment was repeated using third instar, fourth instar, adult male and female *P. dissecta*, as intraguild predator(s). The above experiment was repeated by using ten second-instars (10 h old) of

*M. sexmaculatus* as intraguild prey in a Petri dish. Thereafter, a 1-day-old third-instar *P. dissecta* was released in it as an intraguild predator and the observation on the larval IGP was taken, after 12 hours (n = 10). The experiment was repeated using fourth instar, adult male and female of *P. dissecta*, as intraguild predator(s).

### ***Menochilus sexmaculatus* as an intraguild predator**

The above experiment (as in *P. dissecta* as an intraguild predator) was repeated by switching ten first instars (10 h old) of *P. dissecta* as intraguild prey and second, third, fourth instar, adult male, and female of *M. sexmaculatus* as an intraguild predator(s). Ten numbers of second instars (10 h old) of *P. dissecta* as intraguild prey with second, third, fourth instar larvae, adult male and female of *M. sexmaculatus* as intraguild predator(s).

The data on the IGP of larvae by the two-ladybird species were tested for normality using Kolmogorov – Smirnov Test and homogeneity of variance using Bartlett's Test on statistical software, SAS 9.0 (SAS, 2002). The larval IGP by the different predatory stages were subjected to one-way ANOVA and the data were compared using Tukey's Test using SAS 9.0. IGP by the two species at a particular predatory stage was compared using a two-sample t-test. The data were further subjected to Two-way ANOVA using (i) 'species' and (ii) 'predatory stage' as independent variables and 'IGP' as the dependent variable.

List of abbreviations used: IGP - Intraguild Predation; IG Predator - Intraguild predator; IG prey- Intraguild prey; Ms-*Menochilussexmaculatus*; Pd-*Propylea leadissecta*

## **RESULTS AND DISCUSSION**

All the predatory stages of both ladybird species resorted to IGP of larvae for their survival. However, the intensity of IGP differed significantly between the species. The number of first and second instars consumed by their superior larval stages increased significantly with the larval instars (Table 1). IGP of first (F=4.54; P<0.01; d.f. = 4, 49) and second (F=13.75; P<0.001; d.f. = 3, 39) instars of

*M. sexmaculatus* by predatory stages of *P. dissecta* differed significantly. Similarly, IGP of second instars *P. dissecta* by the older larvae and adults of *M. sexmaculatus* also varied significantly (F=4.91; P<0.01; d.f. = 3, 39). However, IGP of the first instar *P. dissecta* by the predatory stages of *M. sexmaculatus* did not vary significantly (F = 2.33; N.S.; d.f. = 4, 49). Older larval instars and adults of both species consumed the heterospecific inferior larvae, which indicates that perhaps in the fields, older instars might use the early heterospecific instars as food for their survival and development during aphid scarcity. When the IGP was compared at the species level, it was noted that second instar (t = -3.09; P<0.01; d.f. = 12), third instar (t = -2.40; P<0.05; d.f. = 12), adult male (t = -6.50; P<0.001; d.f. = 12) and female (t = -2.60 ; P<0.05; d.f. = 12), *M. sexmaculatus* consumed significantly greater number of first-instars than those consumed by *P. dissecta* (Fig. 1). Similarly, in the case of third instar (t = -3.48; P<0.01; d.f. = 17), fourth-instar (t = -2.54; P<0.05; d.f. = 17), and adult male (t = -4.37; P<0.001; d.f. = 17), *M. sexmaculatus* consumed significantly greater number of second-instars than those consumed by *P. dissecta* (Fig. 2). IGP of second instars by the adult heterospecific females did not differ significantly (t = -1.28; P = 0.219; d.f. = 15). These results support our hypothesis that *M. sexmaculatus* will dominate the agroecosystems, where *P. dissecta* co-occur and will reduce the abundance of latter in the prey absence.

The larval morphology of *M. sexmaculatus* with the presence of spines and hair provides aposematic security against IGP. Dorsal spines of the second instar *M. sexmaculatus* provide physical protection against intraguild predators, as also is the case in *Harmonia axyridis* (Pallas), where spineless larvae were more attacked by superior IG predators than the spined larvae (Hautier *et al.*, 2017). Morphological anti-predator traits and adaptations provide strong selection pressure and defense to the developing stages (Lima and Dill, 1990). Spines are highly conspicuous and offer physical defense in both plants and animals (Inbar and Lev-Yadun, 2005). Defense mechanism due to the presence of

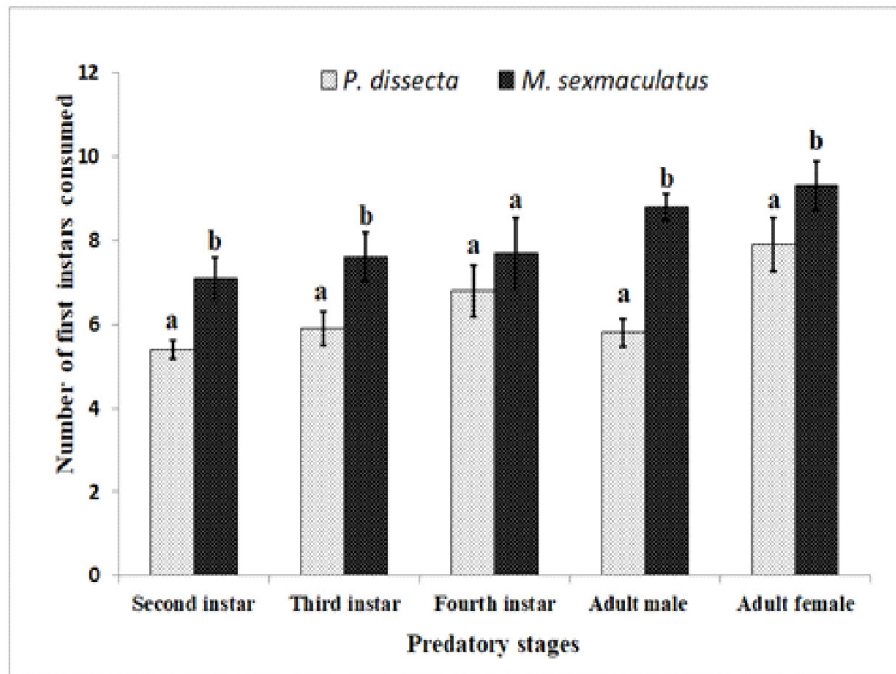


Fig.1 Intraguild predation of the first instar *P. dissecta* and *M. sexmaculatus* by different predatory stages of two ladybirds. Data is Mean  $\pm$  S.E. Different letters denoted in the columns indicate that the data are statistically significant

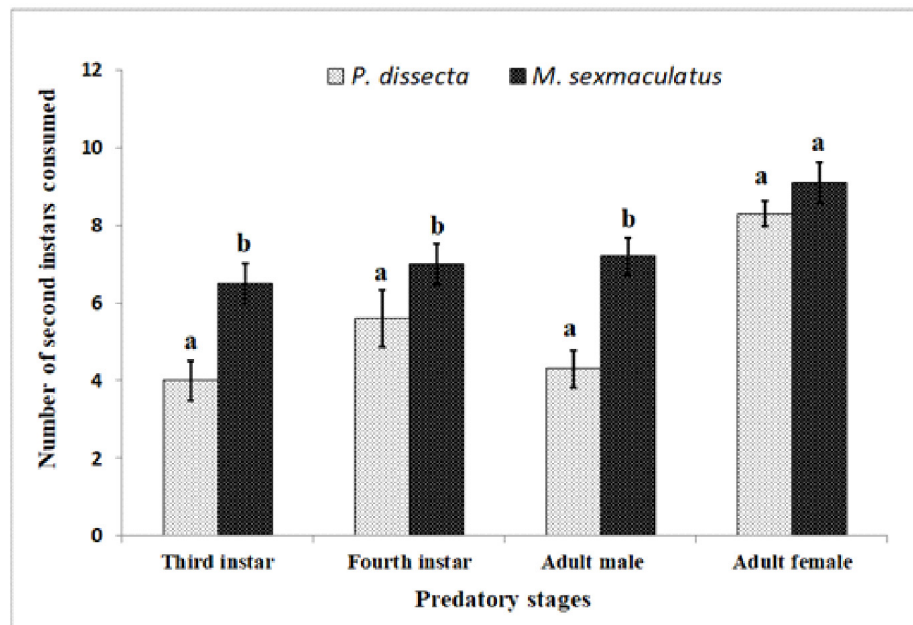


Fig.2 Intraguild predation of the second instar *P. dissecta* and *M. sexmaculatus* by different predatory stages of two ladybirds. Data is Mean  $\pm$  S.E. Different letters in the columns denote that the data are statistically significant

**Table1. Consumption of different predatory stages of ladybirds, *P. dissecta* (Pd) and *M. sexmaculatus* (Ms) on first and second instars**

Prey stage Predator stage	I instar Ms Pd	I instar Pd Ms	II instar Ms Pd	II instar Pd Ms
Second instar	5.4 ± 0.70 a	7.1 ± 1.59 a	-	-
Third instar	5.9 ± 1.28 ab	7.6 ± 1.83 ab	4.0 ± 1.56 a	6.5 ± 1.65 a
Fourth instar	6.8 ± 1.93 bc	7.7 ± 2.71 ab	5.6 ± 2.3 b	7.0 ± 1.7 ab
Adult male	5.8 ± 1.03 ab	8.8 ± 1.03 bc	4.3 ± 1.49 a	7.2 ± 1.47 ab
Adult female	7.90 ± 2.02 c	9.3 ± 1.88 bc	8.3 ± 1.05 c	9.10 ± 1.66 c
F-value	F=4.54;P<0.01*	F=2.33; N.S.*	F=13.75;P<0.001**	F=4.91;P<0.01**

Data represents Mean ± S.D.; \*d.f. = 4, 49 and Turkey's range =4.02; \*\* d.f. = 3, 39; Turkey's range =3.81 estimated using one-way ANOVA. Different letters in the same column denote that the data are statistically significant

dorsal spines perhaps is more needed by the first and second instars, whose smaller size makes them more vulnerable to IG predators. It was noted that dorsal spines perhaps protect larvae against IGP by decreasing the probability of being attacked and increasing the opportunities of biting back or escaping from the IG predators. This could be one of the reasons for the increase in population density and the dominance of *M. sexmaculatus* among the coccinellid fauna in the Oriental region (Omkar and Pervez, 2004). Inferior larval stages of *H. axyridis* secrete noxious reflex blood, harmonine, as a larval antipredator response against the IG predator's attack (Grill and Moore, 1998).

Zarei *et al.* (2020) suggested that size-disparity and hunger level of the IG predators trigger IGP by coccinellids. IGP in favour of *M. sexmaculatus* may be ascribed to its greater body-size and increased activity than *P. dissecta*. However, the fourth instar *M. sexmaculatus* seemed to be sluggish in attacking first-instars *P. dissecta*. This might be due to its critical weight, which might have been achieved before pupation. *Propylea dissecta* also co-occurs with *Coccinella transversalis* Fabricius in the agricultural fields and generally acts as an intraguild prey due to the larger size of the latter (Omkar *et al.*, 2006; Pervez *et al.*, 2006). *Propylea japonica* (Thunberg) was treated as IG prey by the co-occurring *H. axyridis* and

*Coccinella septempunctata* L. (Yang *et al.*, 2017). *Harmonia axyridis* had a higher relative growth rate and faster developmental time during its second and third instar compared to *Coleomegilla maculata* (Labrie *et al.*, 2006). By attaining larger body size, *H. axyridis* is likely to be the successful intraguild predator than *C. maculata*.

Two-way ANOVA revealed that the main effects of 'species' (F = 26.15; P< 0.0001; d.f. = 1) and 'predatory stage' (F = 5.31; P< 0.001; d.f. = 4) were statistically significant using first instars as IG prey. The interaction 'species' and 'predatory stage' was not found to be statistically significant (F=1.04;P=0.390;d.f.=4). Similarly, using second-instars as IG prey, the main effects of 'species' (F = 26.58; P< 0.0001; d.f. = 1) and 'predatory stage' (F = 17.19; P< 0.0001; d.f. = 3) were found to be statistically significant. The interaction 'species' and 'predatory stage' was not found to be statistically significant (F = 1.73; P = 0.390; d.f. = 3). Amongst the predatory stage, the adult female of both the ladybird species consumed a greater number of IG prey, which may be ascribed to the bigger body-size and increased energy demands owing to egg-production (ovariole number) (Rasekh and Osawa, 2020). Thus, both species and the predatory stage have a direct impact on the outcome of IGP. Little is known regarding IGP and egg cannibalism using *M. sexmaculatus* (Agarwala *et*

al., 1998; Agarwala and Yasuda, 2001), however no information is available on its IGP at the larval level. This study seems to be the pioneer in using *M. sexmaculatus* as both intraguild predator and prey. Thus, further studies on IGP and the rate of intraguild predation between the slow and fast developing variants of the two-coccinellid species are needed to investigate its successful establishment and dominance in the agro-ecosystems.

It is concluded that larval instars and adults of *M. sexmaculatus* and *P. dissecta* can attack the lower heterospecific instars during prey scarcity and indulge in IGP. However, *M. sexmaculatus* has more IGP potential, and it could act as intraguild predator in the absence of natural prey, i.e. aphids, and can easily attack heterospecific larvae, which could be the reason for its successful establishment and wide distribution.

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