Influence of intraguild predation between Episyrphus balteatus and Hippodamia variegata on their prey

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Influence of intraguild predation between *Episyrphus balteatus* and *Hippodamia variegata* on their prey

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Intraguild predation (IGP) is an interaction that frequently occurs in natural enemy communities, especially aphidophagous predators. This research investigated IGP intensity between *Episyrphus balteatus* De Geer (Diptera: Syrphidae), with *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae). Five predator combinations including second and third larvae of *H. variegata* and third instar larvae of *E. balteatus* plus control treatment (totally six treatments) were tested. The effect of IGP on cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) population density was investigated on sweet pepper seedlings under laboratory microcosms. In most combinations, the third instar larvae of *E. balteatus* alone reduced an *A. gossypii* population more efficiently than ladybird larvae and their combinations. Furthermore, IGP between third instar of *E. balteatus* and second larvae of *H. variegata* was asymmetrical; second instar *H. variegata* larvae were always the intraguild prey for third instar *E. balteatus*. The obtained result showed that outcome of IGP interaction on cotton aphid density was non-additive.

**Keywords:** ladybird beetle; hoverfly; biological control; inter-specific interaction

**Introduction**

In agricultural systems, understanding food web interactions is often confounded by the complexity of relationships among organisms and by the number of species involved (Bascompte & Melian 2005). There are many interactions in natural systems and one of the most important is that of intraguild predation (IGP). It is a combination of competition and predation that is, killing and eating of species that use similar, often limited resources and are thus potential competitors (Polis et al. 1989; Lucas 2012). IGP significantly affects the distribution, abundance, evolution and population dynamics of those species that are involved and it has become a major research topic in the field of biological control and conservation ecology (Kindlmann & Houdkova 2006).

Organisms feeding on aphids belong to a group known as aphidophagous guild (Rosenheim et al. 1995). Various kinds of interactions are feasible among parasitoids, predators and aphid-specific pathogens (Muller & Godfray 1999; Brodeur & Rosenheim 2000), providing desirable system for IGP. There is a great number of natural enemies with variations in body size during the life cycle, diversity in spatial distribution patterns and enormous density during short-term periods of an aphid’s life cycle that all promote interactions such as IGP among natural enemies (Lucas 2005).
Cotton aphid or Melon aphid, *Aphis gossypii* is a cosmopolitan species widely distributed throughout tropical, subtropical and warm temperate regions. Melon aphid has a very wide host range covering at least 700 host plants known worldwide, some of which are economically important crop for example cucurbits, sweet pepper, eggplant and cotton (Capinera 2001; Blackman & Eastop 2006). The aphid sucks up nutrients, makes leaves curl, distorts and reduces fruit quality and yield. Moreover, melon aphid transmits more than 50 plant viruses including potyviruses (Capinera 2001).

Resistance to insecticide, results in perpetuated outbreaks of this pest and makes its control more difficult (Hollingsworth et al. 1994). Thus, consideration of the use of environmentally friendly control measures to reduce the use of pesticides against cotton aphid is important. Among these alternative methods to the use of pesticides, the use of natural enemies for biocontrol is an obvious choice. The most effective natural enemies against melon aphid known so far are ladybirds, syrphid flies and braconid wasps (Capinera 2001).

*Episyrphus balteatus* (De Geer) (Dip.: Syrphidae) is an efficient aphid predator, larvae of the species are voracious feeders and consume between 400 and 1,000 aphids during their larval development (Tenhumberg 1995; Leroy et al. 2010). Nowadays, *E. balteatus* is the only syrphid species that is commercially available (Pineda & Marcos-Garcia 2008a, 2008b), and several researchers have recently prescribed them as suitable for release with other natural enemies in biological control programmes (Frechette et al. 2007; Pineda et al. 2007).

The variegated lady beetle *Hippodamia variegata* (Goeze) is a well-known aphid predator that has been used in biological control programmes on aphids (Obrycki & Orr 1990; El-Hag & Zaitoon 1996; Obrycki & Kring 1998). It is a Palaearctic species and is an important and naturally abundant natural enemy of *A. gossypii* in Iran (Lotfalizadeh 2001). *H. variegata* is a deserving candidate for natural control, mass rearing and release against different aphid species because of its high population density and high rate of consumption (Franzmann 2002).

In natural ecosystems harbouring rich aphidophagous guild, it is quite common that different aphid natural enemies meet each other while searching for their shared preys and in many cases, these confrontations lead to IGP occurrence. The IGP between ladybirds and *E. balteatus* has been addressed by some researchers (Alhmedi et al. 2007; Putra et al. 2009; Ingels and De Clercq 2011). However, there have been little studies about the outcome of intraguild interactions between *E. balteatus* and other ladybird species like *H. variegata*, especially in more real and complex environments. Thus, the first objective of this study was to measure IGP interaction in laboratory between different immature stages of *E. balteatus* and *H. variegata*. Furthermore, the effect of IGP on cotton aphid population was studied under more realistic conditions.

**Materials and methods**

**Plant and insects rearing**

Cucumber plants, *Cucumis sativus* L. (cv. Negin) were grown individually in regular pots (35 × 30 cm) and used to establish a culture of *A. gossypii*.

Laboratory cultures of predators were started with *H. variegata* and *E. balteatus* that were collected from three locations: alfalfa fields and apricot gardens (Hamedan province, latitude: 34°47’–34°49’N and longitude 49°35’E) and Dorud (Lorestan province). Samples were kept in ventilated containers and brought back to the laboratory.
Adult hoverflies were reared in 60 × 60 × 60 cm net cages and provided with canola pollen, sugar and water placed on Petri dishes on cage floor. Sweet pepper seedlings infested with *A. gossypii* were placed within these net cages for 12 h to allow oviposition. Then, after hatching, syrphid larvae were transferred to Petri dishes and fed daily with cotton aphids.

*H. variegata* adults and larvae were fed with *A. gossypii* in net screened Petri dish (8 cm diameter and 1.5 cm height). Together with aphids, *H. variegata* adults were provided with canola pollen and sugar–water (30% concentration) to improve oviposition. Egg clusters were regularly collected on pieces of paper in Petri dishes. All cultures were kept in growth chamber at 22 ± 2 °C, 50 ± 10% R.H. and 16L: 8D photoperiod.

### IGP experiments

Incidence of IGP and effects of this interaction on the cotton aphid density were studied on potted sweet pepper seedlings with 4–5 leaves. Selected predator stages were single individuals of second and third instar larvae of *H. variegata* and single individuals of *E. balteatus* third instar larvae. In *E. balteatus* third instar larva, is the most voracious life stage. In *H. variegata*, the most voracious fourth instar larvae enter pupal stage after two days, so that second and third instars were selected. The 100 three days old cotton aphid nymphs were placed on sweet pepper seedlings as extraguild prey.

The IGP experiments consisted of six treatments, including single larva of either second or third instar larva of *H. variegata* or a single *E. balteatus* third instar larva and combinations of either instar of the lady beetle with larva of syrphid. In addition, 100 aphid nymphs without the presence of any predator have been used as a control treatment. Ladybird and hoverfly larvae were starved for 6 and 9 h, respectively. The reason for different starvation period comes back to different developmental time of these predators. All treatments were replicated 10 times. After 72 h, the number of live aphids was recorded. Furthermore, presence or absence of predators and their situations on each experimental unit was recorded. The IGP experiments were carried out in the same conditions as above.

### Statistical analysis

Data normality and homogeneity of variance were assessed by Shapiro-Wilcoxon and Levene's test (SAS Institute Inc 2004). Then, the influence of predator treatments on final population of *A. gossypii* was investigated by ANOVA, and Duncan’s multiple range tests was used to compare mean values.

### Results

The presence of predators significantly suppressed cotton aphid populations after 72 h (*F* = 21.34, df = 4, *p*-value < 0.0001). The treatments including third instar larvae of *E. balteatus* or *H. variegata* singly or in combination were more efficient than other treatments. Furthermore, in the presence of *H. variegata* second instar larvae singly or with syrphid larvae, the number of cotton aphid nymphs was significantly higher than other treatments (Table 1).

*E. balteatus* larvae were the more efficient predator relative to *H. variegata* second instars and attacked second instar lady beetle larvae in 6 cases out of 10 replicates.
Therefore, IGP was asymmetrical. In other treatments, no mortality due to IGP has been recorded. Moreover, all of singly applied predators have been found alive at the end of experiment period.

Discussion

The most striking result is that the presence of second instar *H. variegata* somehow decreased the ability of third instar *E. balteatus* to control aphids, compared to what *E. balteatus* did when it was alone.

Our results demonstrate that the IGP outcome between hoverfly larvae and second instar larvae of *H. variegata* was asymmetrical, *E. balteatus* being the intra-guild predator and *H. variegata* the intra-guild prey in most cases. This phenomenon is quite predictable because one of the most important factors affecting an IGP outcome is relative body size of involved predators (Polis et al. 1989; Lucas et al. 2002), whereby smaller species are usually susceptible to becoming prey to hunting predators. Mean sizes of third instar larvae of *E. balteatus* and second instar larvae of *H. variegata* were 9.05 ± 0.8 (Eisner 1971) and 2.28 ± 0.27 mm (Rebolledo et al. 2008), respectively. A single case of smaller aphidophagous predator overpowering a bigger one, described so far, was the lacewing *Chrysopa carnea* third instar larva killing third and fourth instar larvae of ladybird beetle *Harmonia axyridis* (Nedvěd et al. in press).

In addition to being victims during asymmetrical IGP, the defensive strategy exhibited by second instar coccinellid larvae could be used to document this interaction. A secretion of brown fluid from the mouth of the second instar *H. variegata* larvae was observed, and these individuals died after several hours (Tavoosi Ajvad, personal observations).

Adult coccinellids secrete droplets of hemolymph from the tibio-femoral joints of their legs when they are disturbed (Laurent et al. 2005). These fluids contain different repellents and bitter alkaloids (Lebrun et al. 1999; Pettersson 2012) more rarely, such as against larvae of *E. balteatus*, larvae secrete a dark fluid from their mouth as a defensive strategy that may immobilise *E. balteatus* larvae afterward (Hindayana et al. 2001).

Considering the higher density of cotton aphids in the combined application of third instar larvae of *E. balteatus* and second instar larvae of *H. variegata* relative to a single application of *E. balteatus* larvae showed that this reduced effect was particularly related to the occurrence of IGP between those life stages.

It is possible that presence of second instar larvae of *H. variegata* changed the searching behaviour of syrphid larvae, consequently reducing its consumption rate.

Table 1. Number of survived aphids at the various treatments after 72 h (Mean ± SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replication</th>
<th>Mean ± SEM</th>
<th>Predator mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. balteatus</em> (L3)</td>
<td>10</td>
<td>1.1 ± 0.37&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>H. variegata</em> (L3)</td>
<td>10</td>
<td>0.6 ± 0.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>E. balteatus</em> (L3)+<em>H. variegata</em> (L3)</td>
<td>10</td>
<td>0.8 ± 0.49&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><em>E. balteatus</em> (L3)+<em>H. variegata</em> (L2)</td>
<td>10</td>
<td>3.7 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>H. v. (6 larvae from 10)</td>
</tr>
<tr>
<td><em>H. variegata</em> (L2)</td>
<td>10</td>
<td>6 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Control (Aphid only)</td>
<td>10</td>
<td>81.9 ± 1.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Note: Different letter in the same column are significantly different at p<0.01.
IGP could reduce predation pressure on *A. gossypii* in two ways, directly and indirectly. Firstly, ladybirds feeding could be reduced directly as the result of a syrphid larvae attack. Furthermore, searching effort of *H. variegata* second instar larvae may have been decreased in the presence of syrphid larvae. Ladybird second instar larvae are highly sensitive to IGP due to their small size and weaker defence, thus in some cases they moved toward other positions on a plant in order to avoid their superior competitor. Similar observation has been reported for *H. axyridis* and *E. balteatus* (Ingels & De Clercq 2011).

In contrast to second larvae, it was observed that third instar larvae of *E. balteatus* usually avoids contacting with the third instar larvae of *H. variegata* and move to the other parts of a seedling, so that it does not encounter with larger and stronger third instar ladybird larvae. The mean body size of third instar lady beetle larvae is $4.07 \pm 0.45$ mm (Rebolledo et al. 2008). Therefore, the third instar larvae of *H. variegata* is not in danger from attack by third instar larvae of *E. balteatus* in an IGP interaction due to its larger body size, faster mobility and a fully developed defensive mechanism (Tinkeu & Hance 1998).

Other studies about the IGP interactions between *E. balteatus* and different lady beetle species have reported various results (Ingels & De Clercq 2011). Similar to our results, it has been suggested that the presence of *E. balteatus* larvae was significantly inhibited by *Coccinella septempunctata* L. on the pea aphid (*Acyrthosiphon pisum* Harris) infested plants (Alhmedi et al. 2007). Additionally, Alhmedi et al. (2010) reported that *E. balteatus* larvae consumed more *Megoura viciae* Bucktunder under field and laboratory conditions in a short term, but *Harmonia axyridis* larvae were more efficient in a long term. *H. axyridis* often had an advantage over *E. balteatus* in IGP interactions. In these asymmetrical interactions, *E. balteatus* second instar larvae was always the IG-prey for third, fourth and adult *H. axyridis* (Ingels & De Clercq 2011; Nedvěd et al. in press). This discrepancy can be attributed to the involvement of different predatory instars. The hoverfly larvae were superior only as third instars confronting the first instar of *H. axyridis* larvae (Putra et al. 2009). Interactions between *E. balteatus* and *Propylea japonica* larvae tended to result in a size-dependent asymmetric interaction, whereby first and third instar larvae of *E. balteatus* were prey and predator, respectively, when faced against the fourth and first instars of *P. japonica* (Putra et al. 2009). Outcomes of larval interactions between *E. balteatus* and *Scymnus posticalis* were asymmetrical, with *E. balteatus* larvae significantly superior at three out of four combinations, but inferior when the first instar larvae were encountered with the fourth instar of *S. posticalis* larvae (Putra et al. 2009).

In general, these results show that the occurrence of the IGP phenomenon is an important factor in the designation of predator proficiency. Moreover, the results showed that the combination of selected instars between these two predators have not been additive results in the predation on *A. gossypii*. In this research, not all the possible combinations of predators were tested, so more research on this topic needs to be undertaken including the most voracious life stages of *H. variegata* (fourth instar larvae) plus third instar larvae of *E. balteatus* under full natural conditions that may give further insights to their possible combined applications. Additionally, because IGP is a momentary incident and it is difficult to observe and quantify in the field (Lucas 2005), we tried to use microcosm as a semi-natural experimental unit but certainly studying this interaction on a larger scale could produce results that are more realistic.
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