



Interaction between *Podisus maculiventris* and *Harmonia axyridis*, two predators used in augmentative biological control in greenhouse crops

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Abstract. Intraguild predation (IGP) between the pentatomid *Podisus maculiventris* (Say) and the coccinellid *Harmonia axyridis* (Pallas) in the absence or presence of the extraguild prey *Spodoptera littoralis* (Boisduval) and *Myzus persicae* (Sulzer) was studied in the laboratory. Interactions were asymmetric in favor of the pentatomid. *Podisus maculiventris* readily fed upon eggs and larvae of *H. axyridis*, but adult beetles were rarely attacked. Success of attacks by *P. maculiventris* was stage dependent, fourth instars and adults being more successful in killing ladybeetle larvae than second instars. Attacks by *H. axyridis* on the pentatomid were rare and none of them were successful. The effect of introducing extraguild prey on the level of IGP was tested both in petri dishes and on caged sweet pepper plants. When sufficient numbers of *S. littoralis* larvae were present to satiate the pentatomid, predation on *H. axyridis* larvae decreased significantly, indicating that the coccinellid is a less preferred or less vulnerable prey. When the aphid *M. persicae* was presented as extraguild prey, levels of IGP were not altered. Nymphs of *P. maculiventris* successfully completed development when exclusively fed on larvae of *H. axyridis*, but developmental time was longer than on lepidopteran prey. No pentatomid nymphs reached adulthood on aphids alone. IGP by *P. maculiventris* on *H. axyridis* may be of some importance in greenhouse crops, where both predators are being used increasingly in augmentative biological control programs. Nonetheless, it is expected that in practice larger larvae and adults of *H. axyridis* will escape most attacks by the pentatomid.

Key words: Aphididae, augmentation, Coccinellidae, *Harmonia axyridis*, intraguild predation, *Myzus persicae*, Noctuidae, Pentatomidae, *Podisus maculiventris*, *Spodoptera littoralis*

Introduction

In a growing number of biological control programs, several generalist predators are used simultaneously to suppress a complex of pests that may occur

in a given agroecosystem. This may not only lead to competition for prey, but the biocontrol agents may also engage in trophic interaction with one another (parasitism or predation). Intraguild predation (IGP) among generalist predators has been documented to affect the success of biological control (see Rosenheim et al., 1995, for a review). Moreover, generalists may also attack other nontarget species (like pollinators) and especially when exotic species are released in open ecosystems, they may disrupt native arthropod communities. As a consequence of this concern, risk assessment has become a major element in the debate about regulation of macrobial natural enemies for use in augmentative biological control (van Lenteren, 1997; Bolckmans, 1999).

The spined soldier bug, *Podisus maculiventris* (Say), is a generalist predator with a wide host range, attacking more than 90 insect species from eight orders (McPherson, 1980). This predatory pentatomid is native to North America but has been commercially available in Europe since 1997 for control of noctuid pests of greenhouse vegetables and ornamentals, like *Spodoptera exigua* (Hübner) and *Chrysodeixis chalcites* (Esper) (De Clercq and Degheele, 1994; De Clercq et al., 1998a; van Schelt, 1999). Growers have frequently observed that *P. maculiventris* released in sweet pepper crops attacked larvae of the ladybeetles *Harmonia axyridis* Pallas and *Hippodamia convergens* Guérin-Ménéville (J. Klapwijk, Koppert B.V., personal communication); these coccinellids are being used in European greenhouses to suppress outbreaks of aphids (van Schelt, 1999). The polyphagous feeding habit of *P. maculiventris* has led to a growing concern that this pentatomid predator may disrupt the action of other biocontrol agents present in the crop. Evidently, possible side-effects of beneficials should not be disregarded and need to be carefully evaluated, but overestimation of undesired effects based on anecdotal evidence may lead to the unjustified abandonment of an otherwise effective control agent.

Intraguild predation among *H. axyridis* and other coccinellid species has been addressed in a number of studies, including Hironori and Katsuhiko (1997), Cottrell and Yeagan (1998), and Kajita et al. (2000). Predation and competition among *H. axyridis* and *Chrysoperla carnea* (Stephens) were studied by Phoofolo and Obrycki (1998). Predation of *P. maculiventris* on predatory coccinellids has been documented both in the laboratory (Hough-Goldstein et al., 1996) and in the field (e.g. Wheeler, 1977), but the only detailed account on bidirectional interactions between larval and adult life stages of *P. maculiventris* and a ladybeetle (*Coleomegilla maculata* Lengi) was presented by Mallampalli et al. (2002). The current paper focusses on the interactions between different life stages of *P. maculiventris* and *H. axyridis* both in the absence and presence of extraguild prey.

Materials and methods

Insects

A colony of *P. maculiventris* was established in 1999 using specimens originating from a field collection in 1996 near Beltsville, MD. The pentatomid was fed mainly larvae of the greater wax moth, *Galleria mellonella* L., but larvae of the cotton leafworm, *Spodoptera littoralis* (Boisduval), and the yellow mealworm, *Tenebrio molitor* L., were occasionally provided. A colony of *H. axyridis* was started in 1998, using specimens from a commercial source (Biobest NV, Westerlo, Belgium). The coccinellid was fed frozen UV-irradiated eggs of *Ephestia kuehniella* Zeller. Cotton leafworms, *S. littoralis*, used in the experiments were taken from a laboratory colony established in 1994 with insects originally collected in Italy. Larvae were reared on an artificial diet modified from Poitout and Bues (1970). Green peach aphids, *Myzus persicae* (Sulzer), were collected in 1999 in an experimental greenhouse at Ghent University and reared in the laboratory on caged sweet pepper plants (*Capsicum annuum* L., cv. Westlandse Grote Zoete). Colonies of predators and prey were maintained in growth chambers at 23 ± 1 °C, $75 \pm 5\%$ relative humidity (RH), and a 16:8 (L:D) h photoperiod.

Experimental protocol

Five sets of experiments were conducted to determine the interactions between *P. maculiventris* and *H. axyridis*, both in the absence and presence of the extraguild prey *S. littoralis* and *M. persicae*. Experiments primarily aimed at assessing direct trophic interactions between predators. The experiments consisted of: (1) no-choice tests in which individual *P. maculiventris* nymphs or adults were confronted with individual *H. axyridis* larvae; (2) no-choice consumption tests in which individual *P. maculiventris* of different predatory stages were offered a number of *H. axyridis* individuals of each life stage; (3) experiments in which a single *P. maculiventris* nymph was confronted in a petri dish with two *H. axyridis* larvae, in the absence or presence of *S. littoralis* larvae; (4) experiments in which a single *P. maculiventris* nymph and two *H. axyridis* larvae were placed on a potted sweet pepper plant with or without *S. littoralis* larvae; (5) experiments in which a single *P. maculiventris* nymph and two *H. axyridis* larvae were placed on a potted sweet pepper plant with or without *M. persicae* nymphs. An additional set of experiments was carried out to compare the suitability of *H. axyridis* larvae, *S. littoralis* larvae, and *M. persicae* nymphs as food for nymphs of *P. maculiventris*. All experiments were conducted at 23 ± 1 °C, $75 \pm 5\%$ RH and a 16:8 (L:D) h photoperiod.

Individual confrontations

In a first experiment, a single individual of either predator species was placed in a 14-cm diameter petri dish lined with absorbent paper. Interactions between second or fourth instars or adult females of *P. maculiventris* and second, third or fourth instars of *H. axyridis* were tested. Immatures of both species were newly molted (<12 h old); female adults of *P. maculiventris* were reproductively active (>10 days old) and were starved 24 h before testing. Ten replicates were used for each combination of two predators. For each combination, the number of encounters and the number and direction of attacks were monitored during a 90-min period. An encounter was defined as any event in which one individual had detected the other as evidenced by turning, arrestment or vibration of antennae (Heimpel and Hough-Goldstein, 1994; Hodek and Honek, 1996). An attack was considered successful when the attacked individual was subdued and feeding by the attacker was initiated. Level and direction of IGP (i.e. both successful and unsuccessful attacks) were analyzed by log-linear modelling (Agresti, 1990).

No choice consumption tests

Because in the first experiment the interaction between *P. maculiventris* and *H. axyridis* appeared to be mainly unidirectional favoring the former, consumption of the coccinellid by the pentatomid was quantified. In these experiments, individual *P. maculiventris* of various predatory stages were offered a number of individuals of the different life stages of *H. axyridis*. The test arena consisted of a 14-cm diameter petri dish lined with absorbent paper; a soaked paper plug fitted into a 2-cm diameter plastic dish supplied moisture to the pentatomids. Newly molted nymphs of the second to fifth stadium or starved reproductively active females of *P. maculiventris* were placed singly in a petri dish. They were presented with either 35 eggs (in a batch), 10 first instars, 3 second, third or fourth instars, or 3 female adults of the coccinellid. Each combination was replicated 10 times. In order to check mortality of *H. axyridis* in the absence of *P. maculiventris*, 10 control treatments were set up for each life stage of the coccinellid. Survival of *H. axyridis* larvae and adults was assessed after 24 h; eggs were kept until hatching. Mortality percentages in the consumption experiments were corrected for control mortality using Abbott's (1925) formula: $[(\% \text{treatment mortality} - \% \text{control mortality}) / (100 - \% \text{control mortality})] \times 100$.

Choice tests with S. littoralis in petri dishes

Predation on fourth instars of *H. axyridis* by fourth instars or female adults of *P. maculiventris* was investigated in the absence or presence of fourth-instar cotton leafworms. Newly molted fourth instars of *S. littoralis* were chosen as extraguild prey because their body weight was similar to that of fourth instars of the coccinellid (12–15 mg). Tests were conducted in 14-cm diameter petri dishes as above. The following combinations were tested: (1) 2 *S. littoralis* larvae, (2) 2 *H. axyridis* larvae, (3) 2 *S. littoralis* larvae + 2 *H. axyridis* larvae, (4) 1 *P. maculiventris* nymph + 2 *S. littoralis* larvae, (5) 1 *P. maculiventris* nymph + 2 *H. axyridis* larvae, (6) 1 *P. maculiventris* nymph + 2 *S. littoralis* larvae + 2 *H. axyridis* larvae, (7) 1 *P. maculiventris* adult + 2 *S. littoralis* larvae, (8) 1 *P. maculiventris* adult + 2 *H. axyridis* larvae, (9) 1 *P. maculiventris* adult + 2 *S. littoralis* larvae + 2 *H. axyridis* larvae. Each treatment was replicated 10 times. In combinations with cotton leafworms, cubes of artificial diet were supplied. Pentatomids were provided with a moisture source as above. Mortality suffered by *H. axyridis* and *S. littoralis* after 24 h was compared between treatments using a Kruskal-Wallis one-way ANOVA and multiple comparison tests. The experiment-wise probability of type I error was controlled by the Bonferroni method (Sokal and Rohlf, 1995).

Choice tests with S. littoralis on sweet pepper plants

Predation by fourth instar *P. maculiventris* on fourth instars of *H. axyridis* with or without the extraguild prey *S. littoralis* was also assessed on sweet pepper plants (*C. annuum*, cv. Westlandse Grote Zoete). The experimental arena consisted of a plexiglas cylinder (25 cm high, 17 cm in diameter), the top of which was covered with fine mesh screen. Each cylinder contained a single potted sweet pepper plant with 10–15 leaves. The following combinations were tested: (1) 2 *S. littoralis* larvae, (2) 2 *H. axyridis* larvae, (3) 2 *S. littoralis* larvae + 2 *H. axyridis* larvae, (4) 1 *P. maculiventris* nymph + 2 *S. littoralis* larvae, (5) 1 *P. maculiventris* nymph + 2 *H. axyridis* larvae, (6) 1 *P. maculiventris* nymph + 2 *S. littoralis* larvae + 2 *H. axyridis* larvae. Each combination was replicated nine times. The experiment was run for 48 h. After 24 h, dead individuals of *H. axyridis* or *S. littoralis* were replaced. Location of the different insects was recorded after 1, 6, 24, and 48 h. Mortality percentages of *H. axyridis* and *S. littoralis* after 48 h were compared between treatments using Kruskal-Wallis one-way ANOVA and multiple comparison tests with a Bonferroni correction.

Choice tests with M. persicae on sweet pepper plants

In a subsequent set of tests, single fourth instars of *P. maculiventris* were confronted with two *H. axyridis* fourth instars on potted sweet pepper plants either in the absence or presence of *M. persicae*. Test arenas consisted of plexiglas cylinders containing a single sweet pepper plant as described above. For treatments involving aphids, the plants were infested with about 500 late instars of *M. persicae* 3–4 days before the start of the experiment. Because some of the aphid nymphs molted to the adult stage during this period, a mixture of nymphs and adults was present in each experiment. Controls were set up to check survival of the coccinellid larvae in the absence of the pentatomid, both with and without aphids. Each treatment was replicated nine times. The experiment was run for 48 h. After 24 h, dead individuals of *H. axyridis* were replaced. Total mortality suffered by *H. axyridis* larvae after 48 h was compared between treatments using a Kruskal-Wallis one-way ANOVA and multiple comparison tests with a Bonferroni correction. Although predation on aphids by either predator species was monitored, predation rates were not determined.

Suitability of H. axyridis, S. littoralis, and M. persicae as food for P. maculiventris

An additional experiment was set up to compare the suitability of the tested insects as food for *P. maculiventris* nymphs. Because first instars of *Podisus* do not feed and only take up moisture, nymphal development on the different prey was monitored from the second instar on. Newly molted second instars of the pentatomid were isolated in 14-cm diameter petri dishes lined with absorbent paper and supplied with a moisture source. They were provided ad libitum with either *S. littoralis* third instars, *H. axyridis* third instars, or a mixture of *M. persicae* nymphs and adults. Larvae of *H. axyridis* and *S. littoralis* were not supplied with food. Because aphids were presented on a sweet pepper leaf, a control treatment was set up in which the predator nymphs received only a sweet pepper leaf as food. The petiole of the leaf was inserted into an Eppendorf tube filled with water and sealed with Parafilm. Another control group was supplied with last instars of *G. mellonella*, which are considered optimal food for development of *P. maculiventris* (De Clercq et al., 1998b). Development and survival were monitored daily up to the adult stage. Newly emerged female adults were weighed on a Sartorius B120S balance (± 0.1 mg) (Sartorius, Goettingen, Germany). Twenty replicates were done for treatments in which nymphs received animal prey; to evaluate development on sweet pepper leaves only, 10 replicates were set up. Because of lack of normality and of homoscedasticity, developmental times and adult

Table 1. Total number of encounters, percentage of attacks on total number of encounters and percentage of successful attacks between individuals of different stages of *Podisus maculiventris* and *Harmonia axyridis* during a 90-min period in petri dishes

Combination <i>P. maculiventris</i> × <i>H. axyridis</i>	Total number of encounters	Attacks by <i>P. maculiventris</i>		Attacks by <i>H. axyridis</i>	
		% of attacks	% of successful attacks	% of attacks	% of successful attacks
2nd instar × 2nd instar	19	31.5	16.7	10.5	0.0
2nd instar × 3rd instar	12	50.0	0.0	8.3	0.0
2nd instar × 4th instar	27	18.5	20.0	14.8	0.0
4th instar × 2nd instar	10	40.0	75.0	20.0	0.0
4th instar × 3rd instar	34	38.2	23.1	5.9	0.0
4th instar × 4th instar	25	52.0	23.1	8.0	0.0
female adult × 2nd instar	19	47.4	33.3	0.0	0.0
female adult × 3rd instar	12	66.7	50.0	0.0	0.0
female adult × 4th instar	20	60.0	33.3	20.0	0.0

weights were compared between treatments using Kruskal-Wallis one-way ANOVAs and multiple comparison tests.

Results

Individual confrontations

Despite the high number of encounters observed during the 90-min experimental period in all combinations tested, there was no attack by either predator in about 50% of the encounters (Table 1). Nonetheless, analysis of the data by log-linear modelling revealed that overall there were significant differences ($F = 19.37$, $df = 24$, $P < 0.0001$) in the frequencies of the different events (i.e. no IGP, IGP by *P. maculiventris*, or IGP by *H. axyridis*). Over all combinations, the frequency of attacks by *P. maculiventris* on *H. axyridis* was about 5 times greater than the converse ($t = 5.58$, $df = 26$, $P < 0.0001$).

In 6–20% of encounters, coccinellid larvae of the second to fourth stadium attacked second or fourth instars of *P. maculiventris*. Adult pentatomids were only attacked by fourth instars of *H. axyridis*. None of the attacks by larvae of *H. axyridis* on either nymphs or adults of *P. maculiventris* were successful.

Attacks by *P. maculiventris* on *H. axyridis* were observed in all combinations, but success of attacks was considerably lower for second instars than for fourth instars or female adults. When *P. maculiventris* second instars were paired with second to fourth instars of the coccinellid, the frequency of attacks by *P. maculiventris* varied between 18.5 and 50%, but only a few of

Table 2. Mortality (%) of different life stages of *Harmonia axyridis* inflicted by different predatory stages of *Podisus maculiventris* during a 24-h period in petri dishes^a ($n = 10$)

<i>H. axyridis</i> stage	<i>P. maculiventris</i> stage				
	2nd instar	3rd instar	4th instar	5th instar	Female adult
Egg	47.2	41.6	59.7	84.4	22.8
1st instar	32.7	52.0	53.1	74.2	25.0
2nd instar	4.0	28.0	52.0	72.0	64.0
3rd instar	8.6	16.2	54.3	88.6	81.0
4th instar	2.6	27.0	44.4	71.5	87.4
Female adult	0	3.3	0	3.3	0

^aIndividual predators were offered either 35 eggs, 10 first instars, 3 second, third or fourth instars, or 3 adults of *H. axyridis*. Mortality data were corrected for control mortality using Abbott's (1925) formula.

those attacks were successful (0–20%). When fourth instars of the pentatomid were paired with *H. axyridis* larvae of different stadia, attack frequency averaged 38–52%. Three out of four attacks on second instar coccinellids were successful, but rate of success dropped to about 25% when third or fourth instars were attacked. Female adults of *P. maculiventris* attacked *H. axyridis* larvae of the different stadia in 47–67% of encounters and in 33–50% of the cases the coccinellid was successfully subdued.

No choice consumption tests

Eggs of *H. axyridis* were attacked by all predatory stages of *P. maculiventris* (Table 2). Whereas about half of the 35 coccinellid eggs offered were destroyed by second to fourth instars of the pentatomid during a 24-h period, fifth instars killed 84% of eggs. Adults of *P. maculiventris* destroyed only 23% of the number of *H. axyridis* eggs presented, suggesting they had difficulty finding the eggs or feeding on them; alternatively, *H. axyridis* eggs may have been rejected by adults because of their inferior nutritional value. Predation rates on coccinellid larvae of the first stadium were similar to those on eggs. Our observations suggest that most of the second to fourth instars of the ladybeetle successfully evaded attacks by second-instar *P. maculiventris*. Whereas third-instar *P. maculiventris* killed 16–28% of second- to fourth-instar *H. axyridis*, predation rates by fifth instars of the pentatomid increased up to 72–89%. Female adults of the pentatomid killed 64–87% of second- to fourth-instar coccinellids. Predation on *H. axyridis* adults was negligible. Ladybeetle adults either were agile enough to escape or were rejected by the predators after initial contact with the proboscis. In none of the combinations tested was IGP by *H. axyridis* on *P. maculiventris* observed.

Table 3. Interaction between fourth instars of *Harmonia axyridis* and fourth instars or female adults of *Podisus maculiventris* in petri dish arenas with or without fourth instars of *Spodoptera littoralis* ($n = 10$)

Combination ^a	Mortality (%) suffered after 24 h by ^b	
	<i>H. axyridis</i>	<i>S. littoralis</i>
<i>H. axyridis</i> (control)	0 ± 0.0 a	–
<i>S. littoralis</i> (control)	–	0 ± 0.0 a
<i>H. axyridis</i> × <i>S. littoralis</i>	0 ± 0.0 a	55 ± 15.7 b
<i>P. maculiventris</i> nymph × <i>H. axyridis</i>	30 ± 8.2 b	–
<i>P. maculiventris</i> nymph × <i>S. littoralis</i>	–	95 ± 5.0 c
<i>P. maculiventris</i> nymph × <i>H. axyridis</i> × <i>S. littoralis</i>	0 ± 0.0 a	100 ± 0.0 c
<i>P. maculiventris</i> female × <i>H. axyridis</i>	89 ± 11.1 c	–
<i>P. maculiventris</i> female × <i>S. littoralis</i>	–	94 ± 5.6 c
<i>P. maculiventris</i> female × <i>H. axyridis</i> × <i>S. littoralis</i>	75 ± 13.4 c	100 ± 0.0 c

^aA single fourth instar or female adult of *P. maculiventris*, 2 fourth instars of *H. axyridis* or 2 fourth instars of *S. littoralis* were provided per petri dish.

^bMean ± SEM; means within a column followed by the same letter are not significantly different (Kruskal-Wallis test, significance was tested at the $P = 0.05$ level after Bonferroni correction).

Choice tests with *S. littoralis* in petri dishes

Predation frequencies on *H. axyridis* larvae by nymphs and adults of *P. maculiventris* in the absence or presence of the extraguild prey *S. littoralis* are presented in Table 3. No mortality was noted for *P. maculiventris*. Kruskal-Wallis ANOVAs revealed significant treatment effects on mortality of both *H. axyridis* and *S. littoralis* ($H = 29.7$, $df = 5$, $P < 0.0001$ and $H = 25.8$, $df = 5$, $P < 0.0001$, respectively). Mortality of fourth instar *H. axyridis* caused by fourth instars of *P. maculiventris* averaged 30% in the absence of extraguild prey. When *S. littoralis* larvae were present, however, the pentatomid nymphs consumed all caterpillars offered but refrained from preying on ladybeetle larvae. The frequency of predation by female *P. maculiventris* on *H. axyridis* was significantly higher compared to that by nymphs: over a 24-h period, females killed about 90% of the coccinellid larvae. Predation on ladybeetle larvae by female pentatomids was not reduced in the presence of *S. littoralis*, despite high predation rates on extraguild prey. Numbers of extraguild prey available during the experiment were clearly not sufficient to satiate adult *P. maculiventris* and to avoid IGP. When *H. axyridis* larvae were combined with cotton leafworms, the latter suffered 55% mortality, suggesting that the ladybeetle may be responsible for part of the *S. littoralis* mortality in treatments involving all three species.

Table 4. Interaction between fourth instars of *Harmonia axyridis* and fourth instars of *Podisus maculiventris* on potted sweet pepper plants with or without fourth instars of *Spodoptera littoralis* ($n = 9$)

Combination ^a	Mortality (%) suffered after 48 h by ^b	
	<i>H. axyridis</i>	<i>S. littoralis</i>
<i>H. axyridis</i> (control)	0 ± 0.0 a	–
<i>S. littoralis</i> (control)	–	0 ± 0.0 a
<i>H. axyridis</i> × <i>S. littoralis</i>	0 ± 0.0 a	80 ± 8.2 b
<i>P. maculiventris</i> × <i>H. axyridis</i>	78 ± 12.1 c	–
<i>P. maculiventris</i> × <i>S. littoralis</i>	–	84 ± 7.5 b
<i>P. maculiventris</i> × <i>H. axyridis</i> × <i>S. littoralis</i>	34 ± 9.3 b	100 ± 0.0 b

^aA single fourth instar of *P. maculiventris*, 2 fourth instars of *H. axyridis* or 2 fourth instars of *S. littoralis* were provided per plant.

^bMean ± SEM; means within a column followed by the same letter are not significantly different (Kruskal-Wallis test, significance was tested at the $P = 0.05$ level after Bonferroni correction).

Choice tests with *S. littoralis* on sweet pepper plants

Mortality suffered by both *H. axyridis* and *S. littoralis* differed significantly between treatments ($H = 18.5$, $df = 3$, $P < 0.001$ and $H = 21.7$, $df = 3$, $P < 0.0001$, respectively). When single fourth instars of *P. maculiventris* were kept for 48 h on sweet pepper plants together with two fourth-instar *H. axyridis*, the coccinellid suffered on average 78% mortality (Table 4). The level of IGP dropped significantly to 34% when the extraguild prey *S. littoralis* was present. In the experiment combining the three species, all cotton leafworms provided were consumed. Levels of extraguild prey were probably below the satiation level of the *P. maculiventris* nymphs, which may explain why still one third of the coccinellids were preyed upon by the pentatomids. The occurrence of IGP in latter treatment may also be related to competition for prey between both predators. Plant experiments confirmed high predation rates by *H. axyridis* on cotton leafworms found in petri dish assays. When *H. axyridis* was combined with *S. littoralis*, the ladybeetle killed 80% of the cotton leafworms. Rapid depletion of extraguild prey may thus have increased the vulnerability of the coccinellid to IGP by *P. maculiventris*. In none of the combinations was predation on *P. maculiventris* observed.

Overall, cotton leafworms were observed in 60% of cases on the plants. Likewise, *Podisus* nymphs were found in over 65% of cases on the plants, irrespective of the presence of *S. littoralis* or *H. axyridis*. In contrast, *H. axyridis* larvae were only in 30% of cases on the sweet pepper plants; coccinellids were found more frequently on other parts of the experimental arena (i.e. mesh, cylinder). Location of the coccinellids was not influenced

Table 5. Interaction between fourth instars of *Harmonia axyridis* and fourth instars of *Podisus maculiventris* on potted sweet pepper plants with or without *Myzus persicae* ($n = 9$)

Combination ^a	Mortality (%) suffered after 48 h by <i>H. axyridis</i> ^b
<i>H. axyridis</i> (control)	0 ± 0.0 a
<i>H. axyridis</i> × <i>M. persicae</i>	0 ± 0.0 a
<i>P. maculiventris</i> × <i>H. axyridis</i>	74 ± 12.1 b
<i>P. maculiventris</i> × <i>H. axyridis</i> × <i>M. persicae</i>	75 ± 7.4 b

^aA single fourth instar of *P. maculiventris*, 2 fourth instars of *H. axyridis* or ca. 500 late instars of *M. persicae* were provided per plant.

^bMean ± SEM; means followed by the same letter are not significantly different (Kruskal-Wallis test, significance was tested at the $P = 0.05$ level after Bonferroni correction).

by the presence of the other insects. Nymphs of *P. maculiventris* and larvae of *S. littoralis* were observed in about 70% of cases in the top one third of the plant. Larvae of *H. axyridis*, however, did not show a clear preference for the top of the plant, spending on average 46, 26 and 28% of their time in the top, middle and bottom one third of the plant, respectively.

Choice tests with M. persicae on sweet pepper plants

Kruskal-Wallis one-way ANOVA revealed significant differences in mortality of *H. axyridis* between treatments ($H = 28.0$, $df = 3$, $P < 0.0001$) (Table 5). Ladybeetle larvae suffered no mortality when placed on sweet pepper plants with or without aphids. When *H. axyridis* larvae were confined in plant cages with *P. maculiventris*, 74% of the coccinellids were killed. The level of IGP was not reduced when aphids were supplied as extraguild prey. In none of the treatments was mortality of *P. maculiventris* noted. Although predation on *M. persicae* was not measured, *H. axyridis* was repeatedly seen feeding on the aphid. In contrast, nymphs of the pentatomid were not observed to attack *M. persicae* in these experiments.

Suitability of H. axyridis, S. littoralis, and M. persicae as food for P. maculiventris

Nymphs of *P. maculiventris* reached adulthood when fed on *G. mellonella*, *S. littoralis* or *H. axyridis* (Table 6). However, survival on *H. axyridis* was somewhat lower than on lepidopterous prey (70% versus 80–90%). Statistical analyses revealed significant differences among diets in developmental duration of all nymphal stadia of *P. maculiventris* (Kruskal-Wallis one-way ANOVAs, $P < 0.0001$ in all cases). Greater developmental rates on *G. mellonella* than on *S. littoralis* from the fourth instar onwards are probably related

Table 6. Developmental duration (days) and survival of *Podisus maculiventris* on various insect prey or on sweet pepper foliage

Diet	Stadium									
	2nd instar		3rd instar		4th instar		5th instar		Total	
	Duration ^a	n ^b	Duration	n	Duration	n	Duration	n	Duration	n
<i>G. mellonella</i>	4.4 ± 0.2 a	20	3.5 ± 0.1 a	18	3.3 ± 0.1 a	18	6.6 ± 0.1 a	18	17.6 ± 0.3 a	18
<i>S. littoralis</i>	4.5 ± 0.2 a	20	3.1 ± 0.3 a	20	4.4 ± 0.1 b	16	8.4 ± 0.3 b	16	20.3 ± 0.3 b	16
<i>H. axyridis</i>	5.6 ± 0.3 b	16	4.4 ± 0.3 b	16	5.3 ± 0.2 c	16	8.8 ± 0.2 b	14	24.3 ± 0.4 c	14
<i>M. persicae</i>	5.1 ± 0.2 ab	20	7.1 ± 0.5 c	18	18.0 ± 3.6 d	3	–	0	–	–
Sweet pepper foliage	4.1 ± 0.2 a	8	–	0	–	–	–	–	–	–

^aMean ± SEM; means within a column followed by the same letter are not significantly different (Kruskal-Wallis test, significance was tested at the $P = 0.05$ level after Bonferroni correction).

^bNumber of individuals completing the stadium. Experiments with insect prey were started with 20 second instars, the experiment in which only a sweet pepper leaf was provided was started with 10 second instars.

to size differences. Late instars of the pentatomid had difficulty processing third-instar cotton leafworms, which were considerably smaller than seventh instars of the greater wax moth (ca. 5 mg versus 200 mg, respectively) (De Clercq and Degheele, 1994). Despite similarity in size, developmental times on *H. axyridis* third instars were always longer than on *S. littoralis* third instars, except in the fifth stadium. Weight of attained female adults differed significantly among diets ($H = 15.1$, $df = 2$, $P < 0.001$). Females that had developed on wax moth larvae weighed 103.3 ± 5.2 mg (mean ± SEM, $n = 7$). Female predators reared on larvae of *S. littoralis* or *H. axyridis* had significantly lower weights, averaging 59.0 ± 2.9 mg ($n = 9$) and 65.6 ± 1.6 mg ($n = 6$), respectively.

None of the *P. maculiventris* nymphs succeeded to reach the adult stage when presented with *M. persicae* (Table 6). Ninety percent of nymphs completed the third stadium when fed aphids, but only 15% reached the fifth stadium. Developmental duration on aphids was greatly prolonged from the third stadium on. Eight out of ten second instars molted to the next stadium when offered a sweet pepper leaf only. However, all predator nymphs in this cohort died after 5.8 ± 2.1 days in the third stadium.

Discussion

In a laboratory setting, eggs and larvae of *H. axyridis* were readily preyed upon by different stages of *P. maculiventris*, but adults of the ladybeetle were

only rarely attacked. *Podisus maculiventris* was able to complete its nymphal development when fed exclusively on *H. axyridis* larvae, but developmental time was prolonged suggesting that the coccinellid is a suboptimal prey.

When individuals of either predator were placed together in petri dish arenas without refugia during a 90-min period, no IGP was observed in half of the encounters. Success of attacks by *P. maculiventris* was stage dependent: fourth instars and adults were more successful in killing ladybeetle larvae than were second instars. Many *H. axyridis* larvae and adults managed to evade attacks by the pentatomid due to their agility and speed, which corroborates the findings of Hough-Goldstein et al. (1996). For larger ladybeetle larvae counter-attacking also proved to be an efficient defence mechanism. Once caught, however, behavioral and chemical defensive ploys were of very little effect. Many coccinellids are unpalatable to predators because of noxious chemicals that are present both in the haemolymph and the fluid exuded during reflex bleeding (Daloze et al., 1994/1995; Grill and Moore, 1998). Results from our behavioral and developmental studies suggest, however, that defensive chemicals in the haemolymph and reflex fluid of *H. axyridis* larvae are not repulsive or toxic to *P. maculiventris*. On the other hand, attacks on *H. axyridis* adults were rare. Mallampalli et al. (2001) also reported that in their laboratory experiments adults of the ladybeetle *C. maculata* were never preyed upon by nymphs and adults of *P. maculiventris*. Low attack rates on adult beetles could be related in part to unpalatability. Mallampalli et al. (2002) hypothesized that in some coccinellids the concentration of defensive alkaloids may be higher in the adult stage than in the larval stage. Body sclerotization of adult coleopterans may be another reason for low acceptance of this prey type, given that asopine predators have a noted preference for soft-bodied insects (see De Clercq, 2000, for a review). Moreover, in the field adult ladybirds are expected to escape predation by flying away, which they obviously were not capable of in petri dish arenas.

Mallampalli et al. (2002) found that IGP between *P. maculiventris* and *C. maculata* was asymmetric in favor of the pentatomid. Likewise, *H. axyridis* larvae in our study were rarely observed to attack *P. maculiventris* and they were never seen actually feeding on the pentatomid. These attacks were evidently part of a strategy to counteract aggression by the predatory bug.

Experiments in which both predators were kept together in petri dishes or on caged sweet pepper plants for 24–48 h, demonstrated significant predation by *P. maculiventris* fourth instars and adults on fourth instars of *H. axyridis*. In cage experiments without extraguilid prey, the greater spatial complexity did not yield effective hiding places for *H. axyridis* larvae and could therefore not decrease vulnerability of the coccinellid to attacks by *P. maculiventris*. However, experiments in which larvae of the cotton leafworm

S. littoralis were offered as extraguild prey indicate that IGP will decrease significantly when sufficient numbers of caterpillars are present to satiate the pentatomid. These results imply that the generalist *P. maculiventris* has a preference for caterpillars over coccinellid larvae, which is converse to the feeding habits observed in many other Asopinae (Schaefer, 1996; De Clercq, 2000). This hypothesis can be substantiated by conducting prey preference studies, taking possible adaptation to prey used for rearing stock colonies into careful consideration.

When aphids were presented as extraguild prey, levels of IGP were not altered. The presence of aphids did not reduce predation by *P. maculiventris* nymphs on *H. axyridis* larvae. Development tests showed that *M. persicae* was a suitable food only for early instars of the pentatomid. Second instars of *P. maculiventris* reached the fourth stadium on aphids after ~12 days compared to ~8 days on caterpillars, whereas no nymphs succeeded to reach the fourth stadium on sweet pepper leaves only. However, later instars of *P. maculiventris* had difficulty using aphids as food and none of the predators were able to complete nymphal development on aphids alone. Feeding on such suboptimal prey or on plants may have some practical significance because it may help support populations of *P. maculiventris* when preferred prey are scarce (Ruberson et al., 1986; Wiedenmann et al., 1996; De Clercq, 2000). Mallampalli et al. (2002) also reported that the presence of eggs of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), did not eliminate IGP between *P. maculiventris* and *C. maculata*. This can again be explained by the multiple observations that, because of their small size and immobility, insect eggs, including those of *L. decemlineata*, are non-preferred food for nymphs and especially adults of *P. maculiventris* (see De Clercq, 2000, for a review). When foraging, *P. maculiventris* appears primarily attracted to movement of prey and in choice situations between mobile and immobile prey, the pentatomid shows a clear preference for the former.

Both in petri dishes and on caged plants, fourth instars of *H. axyridis* proved to be capable of killing 0.5–1 fourth-instar cotton leafworm per day. As a generalist, this ladybeetle may thus also contribute to the control of some lepidopteran pests, although coccinellids are noted to feed mainly on eggs and early-instar caterpillars (Hodek and Honek, 1996). It should be emphasized, however, that predation rates estimated in the laboratory using unrealistic arenas and insect densities may not be realized in the field (Wiedenmann and O'Neil, 1992).

The problem of IGP between *P. maculiventris* and *H. axyridis* may be pertinent in a greenhouse environment, where both predators are being used together in a growing number of situations. *P. maculiventris* remaining in the crop after suppressing a caterpillar outbreak may switch to alternative prey

like coccinellid larvae and may as such disrupt aphid biocontrol. At recommended release rates of 0.5–2 *P. maculiventris* nymphs and 3–15 *H. axyridis* larvae per plant (Anonymous, 2001), interactions between the two predators are very likely to occur. Nonetheless, the results of this study and of that by Hough-Goldstein et al. (1996) indicate that larger larvae and particularly adults are likely to escape most attacks by the pentatomid. Further, although predation by *P. maculiventris* on *H. axyridis* has repeatedly been observed both in greenhouse and field crops, impact of IGP on the success of biological control has not been assessed under realistic field conditions. In our study, insects were confined at relatively high densities in small artificial arenas. Such laboratory experiments tend to overestimate the killing capacity of a predator and ignore avoidance behavior that may occur at a larger spatial scale. Several arthropod predators have been found to avoid patches where other predators are present, thus reducing IGP, competition, and other indirect interactions (see Janssen et al., 1998, for a review). Therefore, field studies are essential to fully understand the interaction between the two predators.

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