

## ***Orius albidipennis* (Heteroptera: Anthocoridae): Intraguild predation of and prey preference for *Neoseiulus cucumeris* (Acari: Phytoseiidae) on different host plants**

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A widespread interaction in natural enemy populations is intraguild predation (IGP), the intensity and outcome of which may be influenced by several factors. This study examined the influence of host plant characteristics on IGP between *Orius albidipennis* (Reuter) and *Neoseiulus cucumeris* (Oudemans) in laboratory experiments. The intraguild predation between the two predators was bi-directional, but predation by *N. cucumeris* on *O. albidipennis* is presumably of negligible importance. *Orius albidipennis* preyed upon mite eggs and adults in the absence of *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), but in its presence predation on mite eggs was abandoned and predation on adult mites unchanged (sweet pepper) or reduced (eggplant, cucumber). The IGP-level of *O. albidipennis* on *N. cucumeris* was highest on sweet pepper and lowest on cucumber. Inclusion of host plant aspects in evaluations of the IGP potential between predators intended for simultaneous applications for biocontrol is thus of importance.

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## 1. Introduction

Intraguild predation (IGP), defined as killing and eating among potential competitors (Polis *et al.* 1989), is a widespread interaction in natural enemy populations which in recent years has been investigated for a variety of species (Rosenheim *et al.* 1993, Rosenheim *et al.* 1995). IGP, which occurs when one species (the intraguild predator) predate on and competes with another species (the intraguild prey) over a shared prey (extraguild prey), may be uni-directional if just one of the two predator species engages in intraguild predation or bi-directional if both predators predate each other.

IGP may affect the outcome of biological pest control (Colfer & Rosenheim 2001). Especially in greenhouses, it has become common practice to apply several biocontrol agents simultaneously not only against different pest species but also against just one pest species, e.g. parasitoids and gallmidges against aphids, parasitoids and mirids against whiteflies and predatory mites and minute pirate bugs against thrips. In some situations such programmes have been unsuccessful, which by some has been attributed to IGP among the simultaneously applied beneficials and a resulting reduction in biological control efficiency (Myers *et al.* 1989, Rosenheim *et al.* 1993, Hindayana *et al.* 2001, Rosenheim 2005). Thus, Snyder and Ives (2001) showed that generalist carabids interrupted aphid population control by the parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae) in alfalfa; Rosenheim *et al.* (1993) concluded that the effective biocontrol of aphids exerted by lacewing larvae was disrupted by the presence of other predators like damsel bugs and assassin bugs in cotton fields; and Rosenheim (2005) found that *Orius tristicolor* (White) (Heteroptera: Anthocoridae), which alone effectively suppresses spider mite population in cotton fields, often fails to prevent pest outbreaks when other predators like *Geocoris* spp. (Heteroptera: Lygaeidae) are present. These examples illustrate that evaluation of the IGP potential and intensity among biocontrol agents is essential in the selection of appropriate combinations of beneficial species for biological control (Meyling *et al.* 2004).

Different factors may influence on IGP inten-

sity and outcome for instance the relative size, density and growth rate of intraguild and extraguild prey as well as predator aggressiveness, activity and habitat characteristics (Polis *et al.* 1989; Lucas *et al.* 1998; Christensen *et al.* 2002, Muller & Brodeur 2002, Walzer *et al.* 2004). Although host plant characteristics may affect for example prey growth rate (e.g. Madadi *et al.* 2006) and predator mobility (e.g. Rott & Ponsonby 2000a, b) as well as determine the refuge possibilities for the prey (Finke & Denno 2006), the influence of host plants on IGP has only been scarcely investigated (Roda *et al.* 2000). For evaluation of the soundness of combining beneficial species for biocontrol the IGP potential should therefore not just be evaluated on a single host plant, but on several of the plant species for which the beneficial combination is intended to be applied.

In this study we investigated host plant effects on the intraguild predation between two commonly used thrips predators: the minute pirate bug *O. albidipennis* (Reuter) (Heteroptera: Anthocoridae) and the predatory mite *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae), as well as the consequent effect on the number of extraguild prey consumed and the preference between intraguild and extraguild prey.

## 2. Material and methods

All experiments were done on three host plants: cucumber (*Cucumis sativa* L., cv. Viking), sweet pepper (*Capsicum annuum* L., cv. California Wonder) and eggplant (*Solanum melongena* L., cv. Black Beauty). These host plants differ, among other things, in the amount, shape and length of the trichomes on their leaves (Fig. 1). Sweet pepper is void of trichomes, while the trichomes of cucumber are straight and those of eggplant are umbrella shaped and entangled (Fig. 1). Even though the length of the cucumber trichomes and the length of each arm of the eggplant trichomes is approximately the same (ca. 0.5 mm), the shape of the eggplant trichomes result in a larger trichome coverage of the leaf surface, as clearly seen from Fig. 1. For comparison the length of adult *N. cucumeris* is approx. 0.4 mm (Zhang 2003) and that of first instar nymphs of *O.*

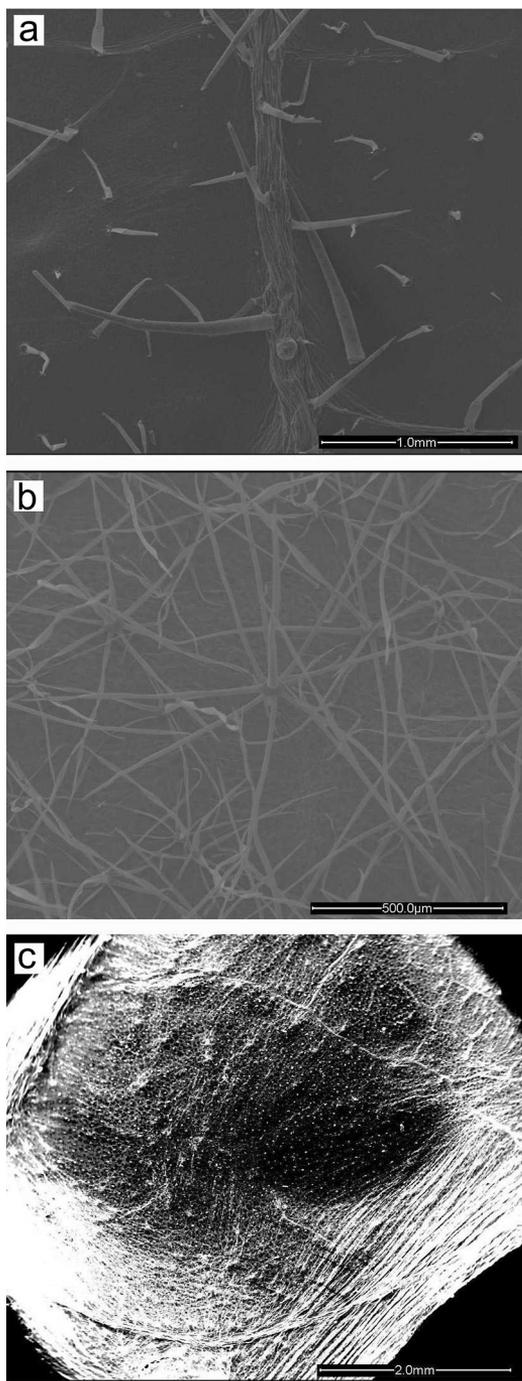


Fig. 1. Scanning electron microscopic photos. – a. Cucumber leaf surface (50 $\times$ ). – b. Eggplant leaf surface (200 $\times$ ). – c. Sweet pepper leaf surface (50 $\times$ ).

*albidipennis* ca. about 0.6 mm (Carnero et al. 1993).

## 2.1. Predators and prey

Onion thrips, *Thrips tabaci* (Lindeman) (Thysanoptera: Thripidae), was reared on bean plants (*Phaseolus vulgaris* L. cv. Montano) in a climate-controlled greenhouse room (25 $^{\circ}$ C, 60% RH, 16:8h L:D). When the two first leaves appeared (three weeks after planting), their petioles were cut and leaves subsequently placed in the small vials (approx 20 ml) filled with water. The vials were placed in Plexiglas containers (19 $\times$ 14 $\times$ 8.5 cm) with lids ventilated with fine mesh net. Synchronised cohorts of thrips were made by placing adult thrips on these leaves and transferring them to new ones after 24 hours. Bean leaves containing thrips eggs were preserved in similar containers for 5 days for the development of second instars, which were transferred to the experiments.

Predatory mites were supplied by EWH BioProduction, Tappernøje, Denmark. Females were taken from the shipments and placed on cucumber leaf discs in small plastic containers (67 mm diameter; 57 ml volume) in a climate cabinet at 25 $\pm$ 1 $^{\circ}$ C, 60 $\pm$ 5% RH, 16:8h (L:D) (prey: mold mites, *Tyrophagus putrescentiae* (Schrank) (Acari: Acaridae)). Mite eggs used for experiments were collected after 12h and positioned randomly on the surface and trichomes of the leaf discs used as experimental units. Adult females used for experiments were max 48h old.

*O. albidipennis*, supplied by Bioplanet, Martorano di Cesena, Italy, was collected in 1999 and 2002 from unsprayed open field zucchini, melon and eggplant in Southwest Sicily (Gino Manzaroli, pers. comm.). After receipt the bugs were reared on Mediterranean flour moth eggs (*Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) supplied by EWH BioProduction) in ventilated glass jars (height 10 cm, diameter 7.5 cm) with leaves of pilea (*Pilea peperomioides* Diels.) supplied as oviposition substrate. Rearing took place in a climate cabinet at the same conditions as above.

## 2.2. Experimental unit

Leaf discs (4 cm diameter) cut from mature leaves of each of the three host plants were chosen as ex-

perimental units. Each leaf disc was positioned upside down on moist filter paper (to prevent thrips larvae from escaping) and placed in a plastic container (67 mm diameter and 57 ml volume) ventilated through a hole in the lid.

### 2.3. Intraguild predation experiments

All the experiments described below were conducted separately on each host plant in climate cabinets at  $25\pm 1^\circ\text{C}$ ,  $60\pm 5\%$  RH. After termination of the experiments the numbers of dead and live individuals were recorded. 15 replicates were made of each experiment and the corresponding control.

Since we suspected that the possible IGP interaction between *N. cucumeris* and *O. albidipennis* would be unidirectional with the latter being the intraguild predator, we first examined if *N. cucumeris* would be able to predate on *O. albidipennis* by testing the predation by the largest and most voracious stage of the mite (adult females) on the most vulnerable stage of the bug (first instar nymphs). Ten adult female mites were placed on each leaf disc together with one first instar *O. albidipennis* nymph. Controls consisted of leaf discs each with just one *O. albidipennis* nymph.

The second part of the experiments examined the predation of adult female *O. albidipennis* on various stages of the predatory mite with or without access to thrips. The following combinations of species were tested in experiments lasting 8h in 8:0h light:dark conditions (experiments 1–3) or 24h in 16:8h light:dark (experiments 4–8):

- 1) One female of *O. albidipennis*, 30 eggs of *N. cucumeris*.
- 2) One female of *O. albidipennis*, 30 eggs of *N. cucumeris*, 50 second instar *T. tabaci*.
- 3) 30 eggs of *N. cucumeris* (control).
- 4) One female of *O. albidipennis*, 40 adult *N. cucumeris*.
- 5) One female of *O. albidipennis*, 40 adult *N. cucumeris*, 90 second instar *T. tabaci*.
- 6) 40 adult *N. cucumeris* (control).
- 7) 40 adult *N. cucumeris*, 90 second instar *T. tabaci* (control).
- 8) 90 second instar *T. tabaci* (control).

### 2.4. Data analyses

All data were tested for normality and non-parametric tests were used whenever data could not be normalised. Prior to analysis observed mortality was corrected for the mortality in the control with Abbott's formula (Abbott 1925).

For analysing the effect of the host plant on the preference of *O. albidipennis* between second instar *T. tabaci* and eggs of *N. cucumeris* Manly's preference index (Cock 1978) was calculated for each host plant and subsequently compared by Fisher-Behrens test with Manly's preference index being:

$$\beta_1 = \frac{\text{Log} \left( \frac{e_1}{A_1} \right)}{\text{Log} \left( \frac{e_1}{A_1} \right) + \text{Log} \left( \frac{e_2}{A_2} \right)} \quad (1)$$

where  $\beta_1$  is the preference to prey type1 (here *N. cucumeris*)  $e_1$  and  $e_2$  are the number of prey type1 and type2 remaining after the experiment,  $A_1$  and  $A_2$  is the number of prey type1 and type2 presented to the predator. If the preference index is close to 1, the predator prefers prey type1 and if close to 0 the prey type2 is preferred. An index value close to 0.5 indicates no preference, i.e. predation is random. Manly's index is the most appropriate in the present case, since it applies to data originating from experiments where killed prey items are not replaced (Cock 1978; Sherratt & Harvey 1993).

## 3. Results

### 3.1. Mortality of *Neoseiulus cucumeris*

Contrary to our expectations, the IGP interaction between *N. cucumeris* and *O. albidipennis* was not completely unidirectional with just the latter species acting as intraguild predator – between 21 and 33% of the first instar *O. albidipennis* nymphs died when exposed singly to 10 adult mites. This mortality was significantly different from the mortality in the control for sweet pepper (Chi-square test:  $\chi^2=6$ ;  $df=1$ ,  $p=0.014$ ) and cucumber (Chi-square test:  $\chi^2=6$ ;  $df=1$ ,  $p=0.014$ ), but not for eggplant (Chi-square test:  $\chi^2=1.934$ ;  $df=1$ ;  $p=0.16$ ). Personal observations (H. Mada-

Table 1. Comparison of the mortality of eggs and adults of *Neoseiulus cucumeris* with the mortality in the controls on the three host plants (Kruskal-Wallis test,  $df=1$ ).

	Sweet pepper		Eggplant		Cucumber	
	$\chi^2$	p	$\chi^2$	p	$\chi^2$	p
Egg	22.7	<0.0001	22.7	<0.0001	22.1	<0.0001
Adult	24.3	<0.0001	23.4	<0.0001	23.7	<0.0001

Table 2. Average mortality of *Neoseiulus cucumeris* expressed as number of killed individuals ( $\pm S.E.$ ) in the different species combinations at three different host plants. The mortality is corrected for the mortality in the control (i.e. *N. cucumeris* alone). Oa = *Orius albidipennis*; Nc = *Neoseiulus cucumeris*; Tt = *Thrips tabaci*. Means within a row followed by the same letter are not significantly different ( $p>0.05$ ). Diff. gives the  $p$ -value for the difference between the means in the two preceding rows.

Combination	Host plant		
	Sweet pepper	Eggplant	Cucumber
Oa + Nc eggs <sup>1</sup>	16.6 $\pm$ 2.6 a	15.9 $\pm$ 1.5 a	10.0 $\pm$ 1.9 a
Oa + Nc eggs + Tt <sup>1</sup>	1.1 $\pm$ 0.5 a	0.4 $\pm$ 0.174 a	0.3 $\pm$ 0.138 a
Diff.	$p<0.0001$	$p<0.0001$	$p<0.0001$
Oa + Nc adults <sup>2</sup>	34.2 $\pm$ 1.05 a	34.0 $\pm$ 0.96 a	24.5 $\pm$ 2.12 b
Oa + Nc adults + Tt <sup>2</sup>	34.6 $\pm$ 0.60 a	26.8 $\pm$ 1.39 b	17.7 $\pm$ 1.42 c
Diff.	$p>0.05$	$p<0.0001$	$p<0.05$

1) The experiments lasted 8h; 2) the experiments lasted 24h.

di) confirmed that these higher numbers of dead *Orius* nymphs were a result of predation from *N. cucumeris* (Fig. 2). Comparison of the number of dead mites observed in this experiment with the mite mortality in experiment 6 revealed significant differences for all host plants (Kruskal-Wallis test: eggplant:  $\chi^2=5.503$ ,  $df=1$ ,  $p=0.019$ ; cucumber:  $\chi^2=9.022$ ,  $df=1$ ,  $p=0.003$ ; sweet pepper:  $\chi^2=6.439$ ,  $df=1$ ,  $p=0.011$ ) indicating that the nymphs of *O. albidipennis* are able to predate on adult mites.

The results of the experiments on the intra-guild predation capacity of adult *O. albidipennis* on predatory mites demonstrated that the pirate bug in the absence of the extraguild prey (*T. tabaci*) preyed upon both eggs and adults of *N. cucumeris* – the mite mortality in combinations with the pirate bug was significantly different from the control (control mortalities ( $\pm s.e$ ): *N. cucumeris* eggs on sweet pepper 0.07 $\pm$ 0.067 %; on egg plant 0.33 $\pm$ 0.21 %; on cucumber 0.13 $\pm$ 0.091; *N. cucumeris* adults: on sweet pepper 0.4 $\pm$ 0.632 %; on egg plant 0.4 $\pm$ 0.632 %; on

cucumber 0.12 $\pm$ 0.368 %) for all host plants (Table 1).

With the extraguild prey present *O. albidipennis* completely abandoned predation of mite

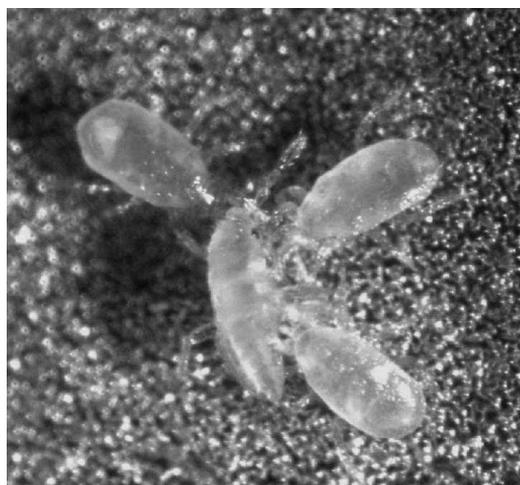


Fig. 2. Predation by three adult *Neoseiulus cucumeris* on a first instar nymph of *Orius albidipennis* (Photo: H. Madadi).

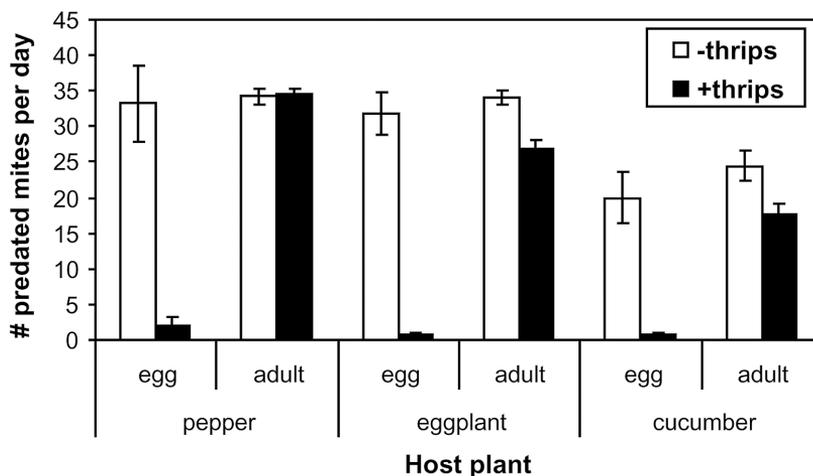


Fig. 3. Mean daily predation ( $\pm$ S.E.) by *Orius albidipennis* on eggs (estimated) and adults (observed) of *Neoseiulus cucumeris* in the presence and absence of extraguild prey *Thrips tabaci* on different host plants.

eggs, the mortality of which was no longer significantly different from that in the control (Kruskal-Wallis test:  $p > 0.05$ ) irrespective of the host plant species (Table 2). However, *O. albidipennis* still displayed its characteristic as a mite predator towards adult mites on all host plants in the presence of thrips (Table 2) with an unchanged predation on sweet pepper (Two-tailed Wilcoxon Mann-Whitney test:  $Z = -0.524$ ,  $p = 0.30$ ) and a significantly reduced predation on eggplant and cucumber ( $t$ -test: cucumber:  $t = -2.67$ ;  $p = 0.013$ ; eggplant:  $t = -4.27$   $p = 0.0002$ ; Table 2).

Direct comparison between the predation of *O. albidipennis* on mite eggs and adults, respectively, is not possible due to differences in the length of the experimental period – however, an indication of the amount of predation on these two mite stages can be obtained by assuming that egg predation would have attained twice the observed values had the experiments run for a full 24h (assuming that *O. albidipennis* only engage in predation under lighted conditions). Fig. 3 presents the thus estimated predation.

Host plant had a significant influence on the intraguild predation by *O. albidipennis* on adults of the predatory mite (Table 2) both in the presence (ANOVA:  $F = 50.21$ ;  $df = 2$ ,  $p < 0.0001$ ) and in the absence of thrips (Kruskal-Wallis test:  $\chi^2 = 15.369$ ,  $df = 2$ ,  $p = 0.0005$ ; Table 2). In the absence of extraguild prey predation on adult mites was lowest on cucumber, and of the same magnitude on the two other host plants, but access to both prey species reduced intraguild predation significantly on eggplant and cucumber, resulting

in significant differences in predation level between all three host plants (Table 2). Contrary to this the intraguild predation on mite eggs, occurring only in the absence of thrips, was not influenced by the host plant species (Kruskal-Wallis test:  $\chi^2 = 5.72$ ,  $df = 2$ ,  $p = 0.057$ ; Table 2).

### 3.2. Thrips mortality

Thrips mortality in the presence of either or both of the predators was in all experiments significantly different from the control (Chi-square test:  $p < 0.001$ ). The mortality imposed on thrips from *O. albidipennis* in the presence of *N. cucumeris* eggs (which were not predated upon) was not significantly different on the three host plants (ANOVA:  $F = 0.66$ ,  $df = 2$ ,  $p = 0.52$ ) while the mortality inflicted by adult predatory mite alone was significantly different (ANOVA:  $F = 27.71$ ,  $df = 2$ ,  $p < 0.0001$ ) being highest on eggplant and lowest on sweet pepper (Table 3).

Thrips mortality in the presence of both predators was only significantly increased on sweet pepper compared to the mortality inflicted by adult predatory mites alone, with the resulting combined predation on thrips being uninfluenced by the host plant species (Kruskal-Wallis test:  $\chi^2 = 2.547$ ,  $df = 2$ ,  $p = 0.28$ ; Table 3).

*O. albidipennis* showed a significant preference ( $t$ -test:  $p < 0.001$ ) for thrips over predatory mite eggs on all three host plants, the preference index ( $\pm$ S.E.) being 0.015 ( $\pm 0.006$ ), 0.039 ( $\pm 0.020$ ) and 0.014 ( $\pm 0.007$ ) for the cucumber,

Table 3. Average mortality of second instar *Thrips tabaci* expressed as number of killed individuals ( $\pm$ S.E.) in the different species combinations at three different host plants. The mortality is corrected for the mortality in the control (i.e. *T. tabaci* alone). Oa = *Orius albidipennis*; Nc = *Neoseiulus cucumeris*; Tt. = *Thrips tabaci*. Means within a row followed by the same letter are not significantly different ( $p>0.05$ ). Diff. gives the  $p$ -value for the difference between the means in the two preceding rows.

Combination	Host plant		
	Sweet pepper	Eggplant	Cucumber
Oa + Nc eggs + Tt <sup>1</sup>	27.2 $\pm$ 2.39 a	30.93 $\pm$ 1.63 a	33.87 $\pm$ 1.76 a
Oa + Nc adults + Tt <sup>2</sup>	63.6 $\pm$ 2.26 a	67.53 $\pm$ 2.97a	68.0 $\pm$ 2.34 a
Nc adults + Tt <sup>2</sup>	35.4 $\pm$ 2.93 a	66.67 $\pm$ 2.17 b	56.7 $\pm$ 2.1 c
Diff.	$p<0.001$	$p>0.05$	$p>0.05$

1) The experiments lasted 8h; 2) The experiments lasted 24h.

sweet pepper and eggplant, respectively, without any differences between host plants (Fisher-Behrens test:  $p>0.05$ ).

## 4. Discussion

### 4.1. Mortality of *Neoseiulus cucumeris*

Our study demonstrated that the intraguild interactions between *O. albidipennis* and *N. cucumeris* were not as unidirectional as anticipated since adult predatory mites were able to prey upon first instars of the pirate bug. However, considering the similarity in size between first instar nymphs of *O. albidipennis* and second instar *T. tabaci* (Fig. 2) combined with the general polyphagous nature of *N. cucumeris* (e.g. McMurtry & Croft 1997) this is perhaps not so surprising after all, although no other study to our knowledge has reported predation by predatory mites on pirate bugs. The magnitude of intraguild predation from *N. cucumeris* on first instar nymphs of the pirate bug was relatively low. In addition, we do not expect that the mites will be able to prey on larger instars of *O. albidipennis* due to their increased size and defence capabilities. Consequently we judge that impact of intraguild predation from mites on pirate bug populations will be negligible.

The present results have, for the first time, demonstrated that adult *O. albidipennis* is an intraguild predator of predatory mites, a characteristic also documented for other species of

*Orius* against mites, as well as against other beneficials like *O. majusculus* (Reuter) (Christensen et al. 2002, Jakobsen et al. 2002, Brødsgaard & Enkegaard 2005); *O. insidiosus* (Say) (Ramakers 1993, Sanderson et al. 2005); *O. laevigatus* Fieber (Wittmann & Leather 1997) and *O. tristicolor* (White) (Cloutier & Johnson 1993). Intraguild predation by *O. albidipennis* on predatory mite eggs only occurred in the absence of extraguild prey, presumably caused by an affinity of *O. albidipennis* for the larger and mobile thrips, whereas adult mites were preyed on both in absence and presence of thrips, although the level of intraguild predation on two of the host plants was significantly reduced in the latter case. Similar reductions in the level of intraguild predation as a result of addition of extraguild prey have also been reported by others (e.g. Sengonca & Frings 1985, Gillespie & Quiring 1992, Cloutier & Johnson 1993, Lucas et al. 1998, Hindayana et al. 2001, Christensen et al. 2002). The level of intraguild predation, as well as the influence of extraguild prey, seen in this study is generally comparable to that reported for *O. tristicolor* preying on *N. cucumeris* (Gillespie & Quiring 1992) and for *O. majusculus* preying upon *Iphiseius degenerans* Berlese (Acarina: Phytoseiidae) (Brødsgaard & Enkegaard 2005).

The host plant species affected the level of intraguild predation of *O. albidipennis* on adult predatory mites but not on mite eggs. The level of intraguild predation was lowest on cucumber, the medium trichomed host plant. This is contrary to our expectations, namely that the dense and long

trichomes of eggplants would offer the highest degree of protection to the predatory mites or, alternatively, impede the movements of *O. albidipennis* the most. Trichome density has been shown by Roda *et al.* (2000) to influence intraguild predation levels on *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) eggs by the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) with a significantly reduced IGP on pubescent apple leaves compared to glabrous leaves.

#### 4.2. Thrips mortality

The level of thrips predation by *O. albidipennis* which seems comparable to its predation on the larger-sized larvae of *F. occidentalis* (Blaeser *et al.* 2004), was uninfluenced by the host plant species, which, however, significantly affected thrips predation by *N. cucumeris*, a phenomenon also described by other authors for the same (Brown *et al.* 1999) or other species of phytoseiids (e.g. Koveos & Broufas 2000, Rott & Ponsonby 2000b). Surprisingly, thrips predation by *N. cucumeris* was lowest on the smooth leaved sweet pepper and highest on the densely trichomed eggplant. Trichomes therefore do not seem to provide refuges for the thrips or to impede the movement of the predatory mites, and other host plant characteristics must be consequently responsible for the observed differences.

Thrips mortality in the presence of both predators did not differ from thrips mortality inflicted by adult predatory mites alone, except on sweet pepper. This result is puzzling in view of the fact that *O. albidipennis* reduced mite populations the most and that *N. cucumeris* preyed the least on this particular host plant. However, the result indicates – together with the fact that sweet pepper is a poor host plant for *T. tabaci* compared with eggplant and cucumber (Madadi *et al.* 2006) – that only on sweet pepper will it be beneficiary to apply both predators compared to relying solely on predatory mites for thrips control.

The present findings indicate that the intraguild predation that becomes possible at a simultaneous application of the two thrips predators may cause a decline in the predatory mite population and thus result in a thrips biocontrol that is

less than additive (sweet pepper) or cancelling out (eggplant, cucumber). However, caution must of course be taken when results from small scale laboratory experiments are used to predict interaction outcomes under greenhouse conditions. Among other things cutting leaves may affect trichome erectness and the chemical composition of the leaves, which in turn may influence the biology of the observed species. Consequently, additional more long termed studies under more practical conditions are needed to further elucidate the practical implications of the intraguild relationship between *O. albidipennis* and *N. cucumeris*.

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