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### Generalist predators, food web complexities and biological pest control in greenhouse crops

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## Biological control of aphids in the presence of thrips and their enemies

G.J. Messelink, C.M.J. Bloemhard, M.W. Sabelis & A. Janssen

Generalist predators are often used in biological control programs, although they can be detrimental for pest control through interference with other natural enemies. Here, we assess the effects of generalist natural enemies on the control of two major pest species in sweet pepper: the green peach aphid *Myzus persicae* (Sulzer) and the Western flower thrips *Frankliniella occidentalis* (Pergande). In greenhouses, two commonly used specialist natural enemies of aphids, the parasitoid *Aphidius colemani* Viereck and the predatory midge *Aphidoletes aphidimyza* (Rondani), were released together with either *Neoseiulus cucumeris* Oudemans, a predator of thrips and a hyperpredator of *A. aphidimyza*, or *Orius majusculus* (Reuter), a predator of thrips and aphids and intraguild predator of both specialist natural enemies. The combined use of *O. majusculus*, predatory midges and parasitoids clearly enhanced the suppression of aphids and consequently decreased the number of honeydew-contaminated fruits. Although intraguild predation by *O. majusculus* on predatory midges and parasitoids will have affected control of aphids negatively, this was apparently offset by the consumption of aphids by *O. majusculus*. In contrast, the hyperpredator *N. cucumeris* does not prey upon aphids, but seemed to release aphids from control by consuming eggs of the midge. Both *N. cucumeris* and *O. majusculus* did not affect rates of aphid parasitism by *A. colemani*. Thrips were also controlled effectively by *O. majusculus*. A laboratory experiment showed that adult predatory bugs feed on thrips as well as aphids and have no clear preference. Thus, the presence of thrips probably promoted the establishment of the predatory bugs and thereby the control of aphids. Our study shows that intraguild predation, which is potentially negative for biological control, may be more than compensated by positive effects of generalist predators, such as the control of multiple pests, and the establishment of natural enemies prior to pest invasions. Future work on biological control should focus on the impact of species interactions in communities of herbivorous arthropods and their enemies.

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**G**eneralist predators are increasingly used to control multiple pests in biological control programs (Chang & Kareiva, 1999; Symondson et al., 2002; Sabelis et al., 2008; Messelink et al., 2010). For example, generalist predatory mites and predatory bugs are among the most successful control agents against common greenhouse pests such as thrips, whiteflies, spider mites and aphids (Gerson & Weintraub,

2007; Sabelis et al., 2008; Cock et al., 2010). An important reason for this success is the ability of these predators to colonize crops when pests are absent or present at low densities because they can feed on alternative food sources. This can result in high predator densities relative to those of the invading prey thereby preventing a pest outbreak. Another reason is that generalist predators can be very effective in suppressing multiple species of plant pests. Several studies have shown that predator-mediated interactions between pest species (apparent competition; Holt 1977) can enhance pest control within a time scale relevant to pest control programs (e.g., Karban et al., 1994; Hanna et al., 1997; Harmon & Andow, 2004; Liu et al., 2006; Messelink et al., 2008, 2010).

However, most generalist predators do not only feed on pests or plant-provided food, but also on other natural enemies, which can be detrimental for biological control (Rosenheim et al., 1995; Rosenheim, 1998; Snyder & Ives, 2001; Symondson et al., 2002; Finke & Denno, 2005; Rosenheim & Harmon, 2006; Janssen et al., 2006, 2007; Lucas & Rosenheim, 2011). This feeding on other natural enemies can be classified as intraguild predation when the enemies share a prey and thus compete for it (Polis et al., 1989; Holt & Polis, 1997; Rosenheim et al., 1995). Predators can also attack other predators with which they do not share a prey, i.e., each predator feeding on a different prey species. Predators consuming other predators has been referred to as 'secondary predation' (Rosenheim et al., 1995), or 'hyperpredation' (Müller & Brodeur, 2002; Messelink et al., 2011), whereas some prefer to use the more general term 'higher-order predation' (Rosenheim, 1998; Symondson et al., 2002). This last definition includes both hyperpredation and intraguild predation. Here, we prefer to use hyperpredation for predators eating other predators without sharing a prey because it has a clear parallel to the term 'hyperparasitism'.

Basic theory about species interactions helps to understand the dynamics of pest-predator interactions, but is often limited to relatively simple systems with only two predators and one prey species (Holt & Polis, 1997). Some recent studies have extended this theory by including food web complexity in the models, such as alternative prey effects (Daugherty et al., 2007; Holt & Huxel, 2007) or spatial heterogeneity (Heithaus, 2001). However, real-life predator-prey systems are often embedded in more complex communities with several interactions among species, and there is no theory for such systems. Many ecologists have recognized this complexity and suggested more empirical studies that test multiple species interactions in realistic natural enemy communities (Rosenheim et al., 1995; Coll & Guershon, 2002; Cardinale et al., 2003; Letourneau et al., 2009). Such studies are of major importance for developing biological control strategies, for example in greenhouse crops where artificial communities are created by releases of several species of natural enemies (van Lenteren, 2000; Enkegaard & Brødsgaard, 2006).

Our main goal is to determine the relative importance of interactions with negative (i.e., hyperpredation and intraguild predation) and positive (i.e., apparent competition) effects on pest control, in a food web of plant pests and their natural enemies. This was studied in a multi-species experiment by assessing the effects of specialist and generalist enemies on the suppression of two major co-occurring pest species in sweet pepper: the green peach aphid *Myzus persicae* (Sulzer) and Western flower thrips *Frankliniella occidentalis* (Pergande). Current biological control programs often fail in suppressing aphids (Bloemhard & Ramakers, 2008) and one reason for this might be that generalist thrips predators interact with specialist aphid natural enemies. Biological control programs for thrips in sweet pepper are usually based on releases of generalist predatory bugs of the genus *Orius* in combination with generalist phytoseiid mites (Shipp & Ramakers, 2004). A common practice for aphid control is the release of a combination of specialised parasitoids (mainly Aphididae) with the specialist predatory midge *Aphidoletes aphidimyza* (Rondani) (Blümel, 2004). The midges are mainly released for controlling aphids, especially when they have reached high densities because specialist parasitoids cannot establish control fast enough. Yet, parasitoids are generally preferred for aphid control at low densities because it is cheaper. Recently, we demonstrated that generalist predatory mites used for thrips control can seriously disrupt biological control of aphids by preying on the eggs of predatory midges (Messelink et al., 2011). Because these predatory mites do not kill aphids, and thus do not share prey with the predatory midges, they can be classified as hyperpredators. In contrast, *Orius* bugs prey on eggs and larvae of *A. aphidimyza* (Christensen et al., 2002; Hosseini et al., 2010), but also on aphids (Alvarado et al., 1997) and therefore act as intraguild predators. Moreover, they are intraguild predators of parasitoids by preying on parasitized aphids (Snyder & Ives, 2003). We compared the effects of these two types of interaction, hyperpredation versus intraguild predation, on the control of thrips and aphids in a setting with the hyperpredator *Neoseiulus cucumeris* Oudemans or the intraguild predator *Orius majusculus* (Reuter) (FIGURE 7.1) together with *A. aphidimyza* and the parasitoid *Aphidius colemani* Viereck. In both food webs, intraguild predation of parasitized aphids by the predatory midge *A. aphidimyza* also occurs (Brodeur & Rosenheim, 2000; FIGURE 7.1). We hypothesized that disruption of aphid control will be stronger with hyperpredators than with intraguild predators, because the hyperpredators only feed on the other natural enemies, whereas the intraguild predators feed on these enemies as well as on the aphids. Moreover, the presence of thrips may contribute to the control of aphids by increasing population densities of the intraguild predators. However, this only applies when the intraguild predators do not have a strong preference for either thrips or aphids. To test this, we observed predation and oviposition rates of *O. majusculus* on both prey when present separately or simultaneously on leaf discs in

the laboratory. These results may help to understand which underlying mechanisms are responsible for effects of different natural enemy assemblages on pest control.

## Material and methods

### Plants, insects and mites

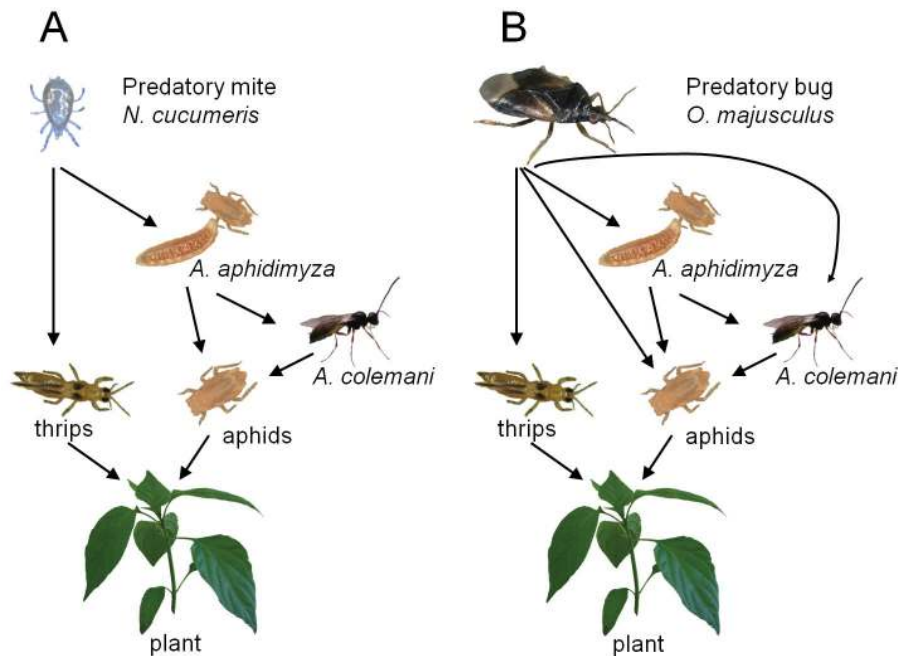
Sweet pepper plants (*Capsicum annuum* L. cv. Spider) were grown by a commercial plant propagator in rock wool blocks in a greenhouse, where they were treated twice with a 0.05% solution of abamectine (Vertimec®, Syngenta) to keep them free of pests. Green peach aphids, *M. persicae*, of the red phenotype (Gillespie et al., 2009) were reared on sweet pepper plants cv. Spider in a greenhouse compartment. Western flower thrips, *F. occidentalis*, were reared on flowering chrysanthemum plants (*Dendranthema grandiflora* Tzvelev, cv. Miramar) in a separate greenhouse compartment. Predatory mites *N. cucumeris*, predatory midges *A. aphidimyza* and the aphid parasitoids *A. colemani* were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands). The predatory bugs *O. majusculus* were obtained from Biobest NV (Westerlo, Belgium). For the prey preference and oviposition experiment, we maintained a laboratory culture of this predatory bug with eggs of the flour moth *Ephesia kuehniella* Zeller as food and bean pods (*Phaseolus vulgaris* L.) as oviposition sites, following methods described by van den Meiracker & Ramakers (1991). The culture was kept in a climate room at 25°C, 70% RH and a photoperiod of 16L:8D. In order to produce second-instar thrips larvae for the laboratory experiment, thrips females were collected from the culture on chrysanthemum and offered fresh bean pods as oviposition substrate, in glass jars, which were closed with lids equipped with a mesh (size 80 µm) to allow ventilation. After 2-3 days the adult thrips were removed and the larvae that emerged from the eggs were grown on the same pods until they reached the second instar. Thrips larvae were reared in a separate climate chamber, under the same conditions as *O. majusculus*.

### Greenhouse experiments

Greenhouse experiments were conducted in a row of six bordering compartments, 24 m<sup>2</sup> each, at the institute of Greenhouse Horticulture (Wageningen UR). The windows of these compartments were provided with insect gauze (mesh size 0.40 × 0.45 mm) to exclude contamination with organisms from outside. Sweet pepper plants cv. Spider were planted in March 2009 in each compartment in four rows, with nine plants per row. Plants were grown according to standard cultivation methods on rock wool slabs with drip irrigation for supplying water and nutrients.

The following natural enemy assemblages were compared: (1) control treatment with releases of only specialist aphid parasitoids and predators (*A. colemani* and *A. aphidimyza*), (2) the hyperpredator *A. cucumeris* together with *A. colemani* and *A.*

*aphidimyza* (strategy A; FIGURE 7.1), and (3) the intraguild predator *O. majusculus* together with *A. colemani* and *A. aphidimyza* (strategy B; FIGURE 7.1). Each treatment was applied in two compartments and each compartment was divided in two fields of 18 plants each. Because the fields were spatially separated by a path between the plant rows, we considered each field as a separate experimental unit. However, some exchange of flying stages of the released species between two fields in one greenhouse compartment might have occurred. The predators *N. cucumeris* and *O. majusculus* were released 4 weeks prior to the pest species on flowering sweet pepper plants of ca. 0.8 m height. The predators can survive and reproduce on such plants because of the presence of sweet pepper pollen as food. This release schedule mimics the situation in commercial greenhouses, where early-season inoculative releases of phytoseiid and anthocorid predators are common practice (Shipp & Ramakers, 2004). *Orius majusculus* was released at densities of 100 adults (60% female) per field (= 5.5 adults/plant), which was repeated after 3 weeks to ensure establishment (TABLE 7.1). The adults were released in the middle of each field. Predatory mites (*N.*



**FIGURE 7.1** – Two strategies for biological control of thrips and aphids in sweet pepper. Arrows indicate consumption of the species at the tip of the arrow by the species at the base of the arrow. Strategy A involves hyperpredation of aphid predatory midges by predatory mites, whereas strategy B involves intraguild predation of aphid predatory midges and parasitized aphids by predatory bugs.

**TABLE 7.1** – Time schedule of pest and enemy releases in greenhouses. The numbers shown are individuals released per field of 18 sweet pepper plants.

	Time (weeks)							
	-3	0	1	2	3	4	5	6
GENERALIST PREDATORS								
<i>Neoseiulus cucumeris</i> <sup>1</sup>	1800							
<i>Orius majusculus</i> <sup>2</sup>	100	100						
PEST SPECIES								
<i>Myzus persicae</i> <sup>1</sup>			36	72	144			
<i>Frankliniella occidentalis</i> <sup>3</sup>			36	36				
APHID ENEMIES								
<i>Aphidoletes aphidimyza</i> <sup>4</sup>					10	20	20	100
<i>Aphidius colemani</i> <sup>4</sup>					6	10	10	20

<sup>1</sup>mixture of juveniles and adults; <sup>2</sup>released as adults, 60% female; <sup>3</sup>adult females; <sup>4</sup>released as pupae, sex ratio 50%.

*cucumeris*) were released once at densities of ca. 100 mites (mixed age) per plant (1800/field) by sprinkling the commercial product (consisting of bran, the storage mite *Tyrophagus putrescentiae* (Schrank) and the predatory mites) on the top of the plants. Release densities were determined by counting the number of predatory mites per gram of product in the laboratory under a binocular microscope (40×), after washing and sieving the material over a 400 µm and 63 µm sieve. Plants were infested three times with green peach aphids *M. persicae* and two times with Western flower thrips *F. occidentalis*, starting 4 weeks after the first releases of *N. cucumeris* and *O. majusculus* (TABLE 7.1). The repeated release served to minimize fluctuations in the densities of thrips and aphids. Individual aphids were transferred from the culture on sweet pepper to the upper leaves of each plant with a fine paintbrush at densities of 2, 4 and 8 per plant respectively during the three consecutive weeks (TABLE 7.1). Thrips were introduced by collecting adult females with an aspirator from the culture on chrysanthemum, and releasing them at a rate of six per three plants (36/field, TABLE 7.1). The specialist natural enemies of aphids, *A. aphidimyza* and *A. colemani*, were released four times at weekly intervals, starting 3 weeks after the first pest introductions. Release densities were higher in the last week because of a strong increase of aphid densities after a few hot days with temperatures above 30°C. The exact release densities of pests and natural enemies per field are presented in TABLE 7.1. Predatory midges and parasitoids were released as pupae and mummies respectively by putting them in a Petri dish with vermiculite (which is the carrier material in bottles of the commercial product), which was placed on the ground in the shade, in the middle of each row of nine plants. Densities of pests and predators were assessed weekly for a period of 7 weeks, starting 4 weeks after the first pest introductions and one week after the last aphid introduction (TABLE 7.1). Population densities of aphids, *O. majusculus*, *A. aphidimyza* and parasitized aphids were fol-

lowed per field by counting the number of individuals of these species on both sides of 10 randomly chosen leaves in the upper plant layer and 10 leaves in a layer that was about 0.5 m below the top of the plant. Mortality of aphids due to parasitism by *A. colemani* was quantified by counting the number of mummies per leaf. These counts were cumulative, because mummies from which the parasitoid had already emerged were not separated from intact mummies. Thrips and predatory mites were more equally distributed on the plants than aphids, and their densities were assessed on eight randomly chosen leaves per field. Because of the smaller size of these organisms, we counted them in the laboratory under a binocular microscope (40×) after picking the leaves in the greenhouse compartments.

Sweet pepper fruits were harvested as soon as they became red. The total production of peppers and the number of peppers severely contaminated by aphid honeydew was recorded per compartment during the entire experiment. Temperature and relative humidity in each greenhouse compartment were registered every 5 minutes throughout the experiment with a climate recorder. Conditions were nearly equal in all compartments, with average ( $\pm$  SE) temperatures of  $21.2 \pm 0.04^\circ\text{C}$  and average relative humidities of  $71 \pm 0.5\%$ . Differences in population dynamics of pests and natural enemies among the treatments were analysed using generalized linear mixed models with time and compartment as random factors to correct for repeated measures and pseudoreplication within compartments. Poisson error distributions were applied for the average numbers of aphids, thrips, mummies and gall midges per leaf per field and a binomial distribution was used for the average fractions of aphid parasitism per leaf per field [parasitized/(parasitized and non-parasitized aphids)]. Effects of treatments on fruit yield and honeydew contamination were analysed with generalized linear mixed models with compartment as random factor to correct for pseudoreplication. A Poisson distribution was applied for the total number of fruits per field and a binomial distribution for the fractions of contaminated fruit per field. Differences among treatments were tested at the 5% level using Fisher's LSD (Least Significant Difference) method. All statistical analyses were performed using the statistical package GenStat Release 13.2 (Payne et al., 2010).

### **Prey preference and oviposition rates of *Orius majusculus***

A laboratory experiment was conducted to determine if *O. majusculus* feeds on thrips as well as aphids when presented together and to assess whether this predator has a strong preference for one of the two prey. This was done because a strong preference could affect pest control in the short term. Simultaneously, we assessed oviposition rates on diets of thrips, aphids and the mixture of the two pests to confirm the assumption that both prey species can contribute to population growth of this predator. The experiment was conducted in a climate room under 16 h of artificial illumi-



nation per day, at 22°C and 70% RH. Predation and oviposition rates were measured with 1-week-old mated females (pre-oviposition period is 4-5 days at 26°C on a diet of *E. kuehniella* eggs; Tommasini et al., 2004), which were starved for one day on bean pods to ensure they were motivated to feed. We used plastic boxes of 5 cm high and a diameter of 6 cm with a sweet pepper leaf disc that was embedded upside-down in water agar (1% agar), making the abaxial side of the discs available to the prey species and predators. Prey was added by infesting the leaf discs with either 80 second instar thrips larvae, 80 third instar aphid nymphs or a mixture of 80 thrips larvae and 80 aphid nymphs, so ample prey was present in all treatments. Each treatment was replicated 11 times. After adding prey to the leaf discs, we included one starved female of *O. majusculus* to each box. The boxes were placed upside down on a tray covered with gauze in order to have the abaxial side of the discs facing downwards as on plants (Ferreira et al., 2008). Ventilation was possible through a hole in the lid covered with insect gauze (mesh size 80 µm). The predatory bugs were transported to a new box with the same densities of freshly added prey after 24, 48 and 72 h. The predation and oviposition rates were measured in these boxes after the predators had been transferred, thus also after 24, 48 and 72 h. Eggs were mainly deposited in the leaf veins and could easily be counted under a binocular microscope (40×). For analysis of oviposition rates, data from the first and second day were omitted to reduce the influence of pre-experimental conditions. Average daily predation and oviposition rates were log-transformed, analysed with standard ANOVA and tested for differences among treatments at the 5% level using Fisher's LSD (Least Significant Difference) method. Analyses were done using GenStat as above.

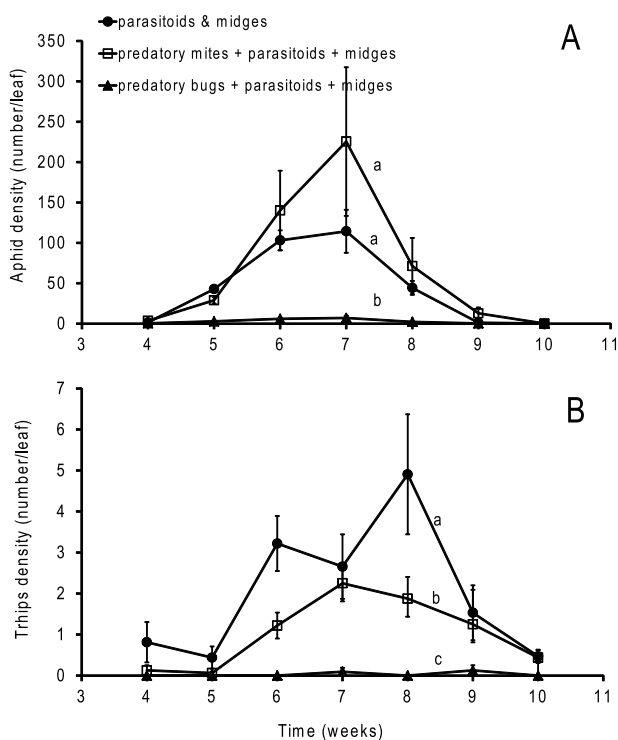
## Results

### Greenhouse experiment

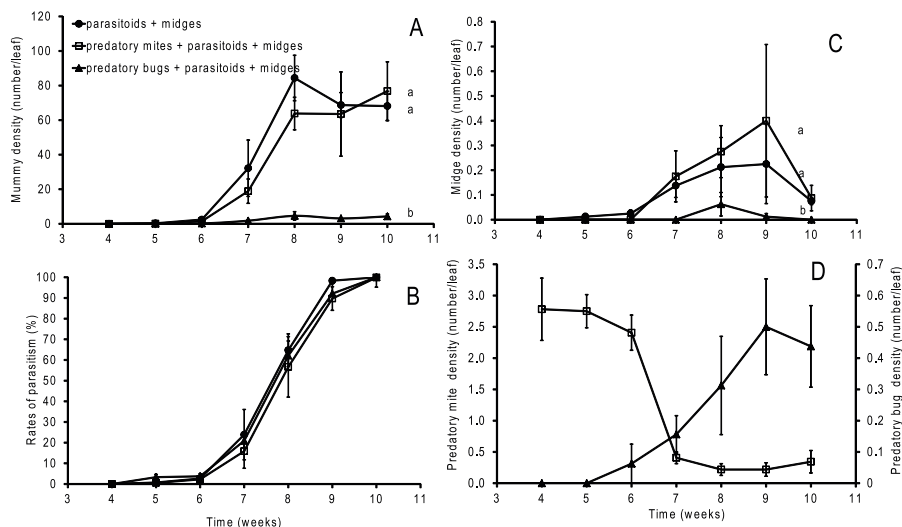
Aphids were effectively controlled in the treatment with predatory bugs + parasitoids + midges, and significantly better than in the treatments with predatory mites + parasitoids + midges or parasitoids + midges ( $F_{2,36} = 5.33$ ,  $p = 0.009$ , FIGURE 7.2A). Aphid densities increased rapidly to high numbers in the latter two treatments. The aphid densities in the treatment with predatory mites, parasitoids and midges were higher than those in the treatment with parasitoids and midges only, but this difference was not significant (FIGURE 7.2A). Overall densities of thrips differed significantly among treatments ( $F_{2,36} = 13.39$ ,  $p < 0.001$ ) and the best control was achieved in the treatment with predatory bugs plus the specialised aphid enemies (FIGURE 7.2B).

Eventually, all aphids were parasitized by *A. colemani* in all treatments in the last week of the experiment (FIGURE 7.3A, B). Numbers of mummies in the treatment with predatory bugs were significantly lower than in the other treatments ( $F_{2,36} = 3.62$ ,  $p = 0.037$ ; FIGURE 7.3A), but the percentages of parasitism were not different among

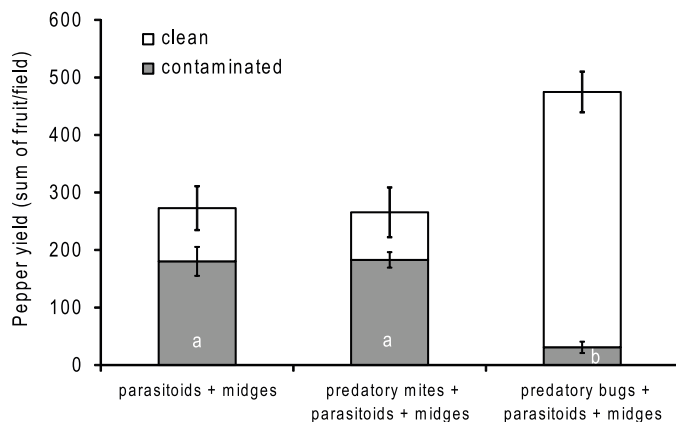
treatments ( $F_{2,36} = 0.06$ ,  $p = 0.94$ ; FIGURE 7.3B). Densities of midges were significantly lower in the treatment with predatory bugs than in the other two treatments ( $F_{2,33} = 5.61$ ,  $p = 0.008$ ; FIGURE 7.3C). Predatory mite densities suddenly dropped to low number between 6 and 7 weeks after the first pest introductions, whereas densities of predatory bugs continued to increase during the whole experiment (FIGURE 7.3D). The better aphid control in the treatments with predatory bugs resulted in a significantly lower percentage of fruits contaminated with honeydew ( $F_{2,3} = 32.58$ ,  $p = 0.004$ ; FIGURE 7.4). Fruit yield was not significantly different among treatments ( $F_{2,3} = 4.68$ ,  $p = 0.12$ ). Slight silver damage on the fruits, caused by thrips, was found only occasionally and therefore not quantified.



**FIGURE 7.2** – Population dynamics of (A) the green peach aphid *Myzus persicae* and (B) Western flower thrips *Frankliniella occidentalis* in a sweet pepper crop in the presence of three assemblages of natural enemies. All three treatments received parasitoids (*Aphidius colemani*) plus predatory midges (*Aphidoletes aphidimyza*). The generalist predatory mite *Neoseiulus cucumeris* (treatment predatory mites + parasitoids + midges) or the generalist predatory bug *Orius majusculus* (predatory bugs + parasitoids + midges) were furthermore released in two treatments prior to the aphid enemies (see TABLE 7.1 for release rates and times). Shown are average ( $\pm$  SE) densities per leaf. Different letters indicate significant differences among treatments through time (Fisher's LSD test,  $p < 0.05$ ).



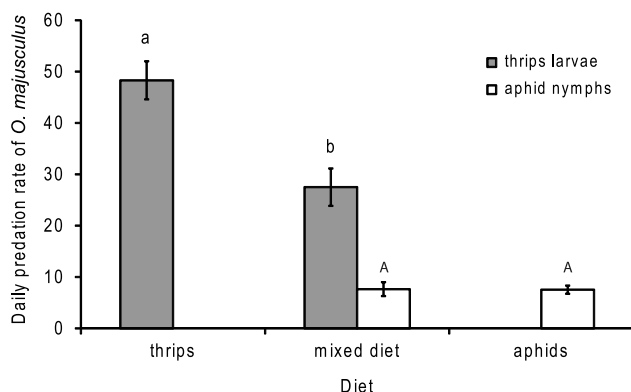
**FIGURE 7.3** – Population dynamics of (A, B) the parasitoid *Aphidius colemani*, (C) the predatory midge *Aphidoletes aphidimyza* and (D) the predatory mite *Neoseiulus cucumeris* and the predatory bug *Orius majusculus* in a sweet pepper crop infested by the green peach aphid *Myzus persicae* and Western flower thrips *Frankliniella occidentalis*. See legend to FIGURE 7.2 for further explanation. Shown are average ( $\pm$  SE) percentages of parasitized aphids and average ( $\pm$  SE) densities of mummies, midge larvae and predators per leaf. Different letters indicate significant differences among treatments through time (Fisher's LSD test,  $p < 0.05$ ).



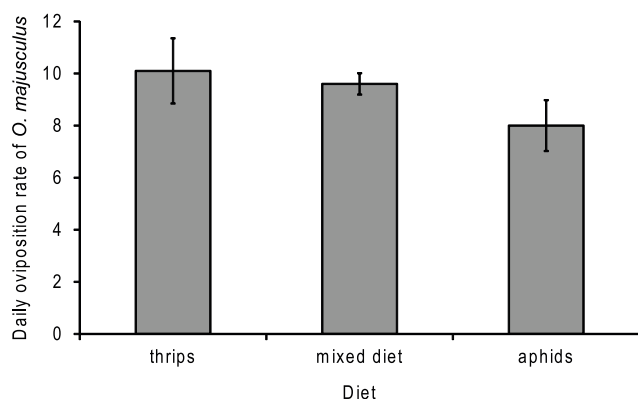
**FIGURE 7.4** – Total number ( $\pm$  SE) of clean and honeydew-contaminated pepper fruits from plants infested with the green peach aphid *Myzus persicae* and Western flower thrips *Frankliniella occidentalis* in the presence of three assemblages of natural enemies. Fruit production was measured during 18 weeks. See legend to FIGURE 7.2 for further explanation. Different letters within bars indicate significant differences in contamination with aphid honeydew among treatments (Fisher's LSD test,  $p < 0.05$ ).

### Prey preference and oviposition rates of *Orius majusculus*

All females of *O. majusculus* consumed aphids as well as thrips when these two prey species were offered together, showing that they do not exclusively prefer either of the two prey (FIGURE 7.5). The consumption of thrips larvae was significantly lower (43%) in the presence of aphids ( $F_{1,19} = 13.39$ ,  $p = 0.002$ ), whereas the consumption of aphids was not significantly changed by the presence of thrips ( $F_{1,20} = 0.11$ ,  $p = 0.74$ ). The predatory bugs produced eggs on all diets of prey (FIGURE 7.6), and oviposition rates after 72 h did not differ significantly among the three diets ( $F_{2,30} = 1.26$ ;  $p = 0.30$ ).



**FIGURE 7.5** – Number of prey consumed by one-week-old adult females of *Orius majusculus* per day when offered second instar thrips larvae and third instar aphid nymphs either separately or in combination (mixed diet). Shown are average numbers of prey consumed ( $\pm$  SE) per female per day (measured over 3 days). Different letters above bars indicate significant differences in consumption of thrips or aphids between the mixed pest treatment and the single pest treatment (Fisher's LSD test,  $p < 0.05$ ).



**FIGURE 7.6** – Average daily oviposition rates of 10-days-old adult female *Orius majusculus* when offered either thrips larvae and aphid nymphs separately or in combination (mixed diet). Shown are average numbers of eggs ( $\pm$  SE) per female per day.

## Discussion

We aimed to assess the impact of generalist predators involved in intraguild predation or hyperpredation on specialised natural enemies, herbivore densities and the yield in a sweet pepper crop. The hyperpredator *N. cucumeris* and intraguild predator *O. majusculus* were both expected to release aphids from control because both predators prey on the specialised natural enemies of the aphids. However, the addition of *O. majusculus* to predatory midges and parasitoids clearly improved the control of aphids. Thus, intraguild predation by *O. majusculus* on predatory midges and parasitoids did not release the aphids from control. Apparently, these effects of intraguild predation were outweighed by the extent to which *O. majusculus* preyed upon aphids. As expected, the hyperpredator *N. cucumeris* did not affect aphid densities significantly. This corresponds with an earlier study, where *N. cucumeris* also did not significantly disrupt aphid control (Messelink et al., 2011). However, hyperpredation by the predatory mite *Amblyseius swirskii* Athias-Henriot on predatory midges clearly disrupted the biological control of aphids (Messelink et al., 2011). Yet, caution should be exercised, because the effects of hyperpredation may depend on the densities of the predatory mites (Messelink et al., 2011). Not only aphids, but also thrips were strongly suppressed by *O. majusculus*. Aphids and thrips were ultimately controlled in all treatments, but the lower aphid densities in the treatments with predatory bugs significantly decreased the number of honeydew-contaminated fruits. The reason why thrips densities ultimately also went down in the treatment without thrips predators is not clear. The high aphid densities in this treatment possibly reduced plant quality and consequently the reproduction rate of thrips.

The results of our study do not provide evidence for strong negative or positive effects of the generalist predators on parasitoids; the rates of parasitism were not affected by the presence of both the predatory mites or predatory bugs. Possibly, such effects were not detected because of the repeated releases of adult parasitoids, which are invulnerable to predation by predators. However, females of *A. colemani* live relatively short (ca. 10 days) and most eggs are laid within the first 3 days after emerging from mummies (van Steenis, 1993). Hence, we assume that the observed parasitism in the 5 weeks after the last parasitoid release was caused by the offspring of the released parasitoids, and these parasitoids had been exposed to intraguild predation. Although rates of parasitism were not different among treatments, the absolute numbers of parasitized aphids were much lower in the treatments with predatory bugs compared to the other treatments, likely because the number of aphids available for parasitism was also lower as a result of aphid consumption by the predatory bugs. However, the predatory bugs probably also consumed parasitized aphids. Because equal numbers of parasitoids were released in

all treatments, the ratio parasitoid: aphid was higher in the treatments with predatory bugs because of the lower number of aphids. Thus, higher rates of parasitism were expected in the treatment with predatory bugs. This was not observed, perhaps as a result of intraguild predation of parasitized aphids by the predatory bugs. However, parasitoids may also have been less effective at these lower aphid densities because they had to spend more time on host searching.

One explanation for the excellent aphid control in the greenhouse compartments with *O. majusculus* is that the presence of thrips and midges might have increased the densities of *O. majusculus*, which consequently increased predation on aphids. This so-called predator-mediated apparent competition between prey species can enhance pest control (Karban et al., 1994; Messelink et al., 2008). Similar mechanisms were recently found by Yoo & O'Neil (2009), who showed that thrips promote colonization of soybean fields by *O. insidiosus* prior to the arrival of soybean aphids and that this resulted in low levels of aphids. In addition to these prey, the pollen from sweet pepper flowers probably also contributed to the establishment of the predatory bugs. The presence or absence of pollen may also affect the intensity of intraguild predation by predatory bugs (Shakya et al., 2009), but because sweet pepper plants flower continuously, pollen supply did not vary in our experiments.

Besides the positive effects of thrips on the predators, we cannot exclude the possibility that the presence of thrips released aphids from control by predatory bugs in the short term (Desneux & O'Neil, 2008), because we did not collect data during the first 4 weeks. Such an effect might even be stronger when the predatory bugs prefer thrips to aphids as prey (Desneux & O'Neil, 2008). However, our laboratory experiment shows that adult predatory bugs did not exclusively prefer either of the two prey species; consumption of aphids was even not affected by the presence of thrips. Furthermore, the predatory bugs produced eggs on diets of both prey species. Thus, the presence of thrips probably contributed to the control of aphids because it resulted in higher densities of predatory bugs. Such effects of apparent competition can even be amplified by a positive effect of mixed prey diets on the predator's reproduction rate (Messelink et al., 2008). However, we found no evidence for such effects, but perhaps the duration of the experiment was too short to observe differences in reproduction.

The opposite effect, the presence of aphids resulting in a release thrips from control might also have occurred in the short-term, because the laboratory experiment showed that the presence of aphids reduced predation of thrips by the predatory bugs. This might have occurred in the first 4 weeks after the first pest releases, i.e., during the initial period when no data were collected. However, the low thrips densities after 4 weeks and the absence of significant crop damage by thrips suggests that, if present at all, such an effect was not strong.

Increased densities of *O. majusculus* through predation on thrips and aphids might have increased the effects on the intraguild prey (parasitized aphids and midge eggs and larvae). Indeed, midge densities were lowest in the treatment with predatory bugs, and this could have been caused through predation of midges by predatory bugs and by competition between bugs and midges for aphids. Thus the decreased densities of midges might have released aphids from control by this predator, but this effect was apparently less strong than the direct negative effect due to predatory bugs consuming aphids.

Equilibrium theory on intraguild predation predicts that disruption of biological control only occurs when the intraguild prey is the better competitor for the shared pest than the intraguild predator (Holt & Polis, 1997; Janssen et al., 2006, 2007). Although these predictions may not directly apply to dynamics at a shorter time scale (Briggs & Borer, 2005), it is possible that the intraguild predator used here (*O. majusculus*) was simply a better competitor for aphids than the intraguild prey (parasitoids and midges). In that case, theory predicts that the intraguild prey should be outcompeted by the intraguild predator, and indeed, the midges tended to disappear in the treatment with predatory bugs (FIGURE 7.3B).

Several studies with generalist predators found that predation rates increased in the presence of multiple prey species (Lucas et al., 2004; Madsen et al., 2004; Koss et al., 2004). Our laboratory experiment possibly indicates such effects for *O. majusculus*. Although predation rates on thrips decreased in the mixed diet, predation on aphids did not change compared to that on a diet of aphids only. Thus, the total number of prey killed increased in the mixed diet relative to the diet of aphids only. This effect cannot be a result of simply more prey in the mixed diet, because ample prey was offered in all treatments.

So far, the biological control of aphids in greenhouses is mainly based on releases of specialised natural enemies (Ramakers, 1989; Blümel, 2004), perhaps based on criteria for selecting natural enemies that were advocated in the past (van Lenteren & Woets, 1988). However, the results of our study suggest that generalist predatory bugs, although potentially risky as intraguild predators, can play a major role in controlling aphids. They are able to respond rapidly to aphid infestations because of their continuous presence in a crop. One could argue that sufficient densities of these predators would even suffice to control aphids. However, inoculative releases of predatory bugs might in some cases not be sufficient for suppressing high aphids densities because the generation time of predatory bugs is too long for a timely numerical response. In such cases, it might be better to additionally release enemies with a strong numerical response, such as parasitoids. Specialised aphid predators that can 'clean up' dense aphid colonies, such as predatory midges, may additionally be necessary to control aphids.

A central question of this study was whether hyperpredation or intraguild predation is more risky for biological control. Hyperpredators mediate an indirect interaction between the alternative prey (thrips in our case) and the specialist predator (predatory midges in our case, FIGURE 7.1). This interaction can be classified as apparent competition, because the two prey species interact through a shared hyperpredator population (Holt, 1977), but with the two prey of the hyperpredator occupying different trophic levels. Theory on apparent competition predicts that the presence of one prey lowers the equilibrium densities of the second prey. For hyperpredation, this would mean lower equilibrium densities of the specialist predator, which could consequently release the prey of the specialist from control. Thus in general, it is expected that hyperpredators will decrease the densities of specialist predators that are vulnerable for hyperpredation, and consequently increase the densities of the prey of these specialists. The reason we did not find a significant reduction of midge densities by the hyperpredator *N. cucumeris* in our study, may stem from fact that the high aphid densities caused contamination of the leaves with sticky honeydew, which may well have reduced predatory mite activity (Nomikou et al., 2003). Preliminary results from a laboratory experiment showed that the presence of sticky honeydew hinders predatory mite movement and strongly reduced predation rates on thrips (measured after 24 h, G.J. Messelink, pers. obs.). As discussed above, intraguild predation by predatory bugs on parasitoids and midges did not affect aphid control negatively. This corresponds with previous studies showing that intraguild predators may reduce densities of intraguild prey, but in general do not disrupt control of the shared prey (Janssen et al., 2006, 2007; Vance-Chalcraft et al., 2007).

In conclusion, our study shows that potential negative effects of intraguild predation on biological control may be compensated by positive effects, such as the control of multiple pests by generalist (intraguild) predators, and the establishment of these predators prior to pest invasions. Thus, research on biological control should assess the impact of generalist predators in relevant pest-natural enemy communities.

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