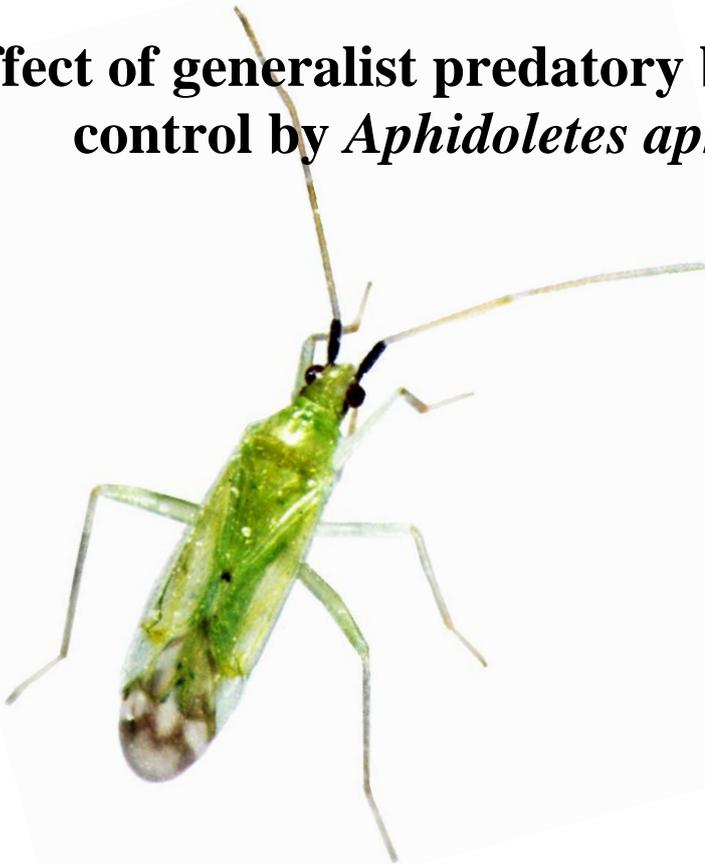




Effect of generalist predatory bugs on aphid control by *Aphidoletes aphidimyza*



No 09.01

NameRenske Vellekoop

Period ½ Aug – Nov 2008

Thesis/Internship ENT Thesis

1^e Examiner Ir. Gerben Messelink

2^e Examiner Prof. Dr. Marcel Dicke

**Thesis Entomology
09.01**

By Renske Vellekoop
Stud.nr: 850815864080

½ Aug - November 2008 (24 ECT)

Examinators:

Ir. Gerben Messelink from *Plant health, WUR Glastuinbouw Bleiswijk*
Prof. Dr. Marcel Dicke of the *Entomology Department, Wageningen University*

Picture on front page: The generalistic predatory bug *Macrolophus caliginosus*.

Abstract

Aphids belong to one of the most serious pest species of greenhouse crops. Natural enemies as *Aphidoletes aphidimyza* can be effective control agents against this pest species and are widely used in pest control. However, the problem exists that in certain situations this natural enemy of aphids can't establish easily in the crop. Intraguild predation (defined as two species that share a host or prey also engage in a trophic interaction with each other) among aphidophagous control agents could be involved. In this study it was examined (i) to what extent the generalist predatory bugs *Macrolophus caliginosus*, *Orius laevigatus* and *Orius majusculus* affect biological control of aphids by *Aphidoletes*, (ii) if alternative prey play a role in these IGP interactions and (iii) if prey preference or predator switching affect IGP. The results of this study demonstrate the presence of intraguild predation although it seems to have a low impact on aphid control by gallmidges on the short term. The presence of alternative food for the predatory bugs is not found to affect IGP interactions although it is found to influence aphid development. Further research is required to support the suggested underlying mechanisms (i.e. plant-mediated/trait-mediated indirect interactions). Gallmidges as additional food source, seem to enhance *Orius* development which may be beneficial for thrips control although not for aphid control (increased IGP on gallmidges). As IGP by predatory bugs does not seem to affect *Aphidoletes aphidimyza*, its bad establishment may be caused by other predators (e.g. predation by mites) or have to do with environmental factors, leaving clearance for further research.

Content

Abstract	3
Content	4
1 Introduction.....	5
1.1 General introduction	5
1.2 Problem description and objectives	6
1.2.1 Pest species: <i>Myzus persicae</i>	6
1.2.2 Specialist predator: <i>Aphidoletes aphidimyza</i>	6
1.2.3 Intraguild predation	7
1.3 Aim of the study.....	10
2 Material and methods.....	11
2.1 Rearing and collecting of prey and predators.....	11
2.2 Experimental design.....	12
2.2.1 Effect of <i>M. caliginosus</i> on aphid control by gallmidges.	12
2.2.2 Effect of Orius spp. on aphid control by gallmidges.	14
2.2.3 Prey preference of the anthocorid bugs.	16
2.3 Statistical analysis.....	17
3 Results.....	18
3.1 Effect of <i>Macrolophus caliginosus</i> on aphid control by gallmidges.....	18
3.2 Effect of anthocorid bugs on aphid control by gallmidges	20
3.2.1 Prey preference	20
3.2.2 Greenhouse experiment	21
4 Discussion	23
5 Conclusions.....	25
Recommendations	26
Acknowledgements	27
References	28

1 Introduction

1.1 General introduction

Aphids belong to one of the most serious pest species of greenhouse crops (Rabasse & Steenis, 2000; Yano, 2006). Many aphid species occurring in the field can become greenhouse pests as the plant conditions and climatic factors are often optimal for development and reproduction. Alate individuals enter the greenhouse through the vents and start invading the crop. Due to their high reproduction capacity high numbers of aphids develop in a short period of time. They suck up plant fluids to obtain nutrients, disturbing the growth hormone-balance of the plant (Malais & Ravensberg, 2002). Having the ability to attain very high densities on young plant tissue, aphids can cause water stress, malformed leaves, wilting and a reduced growth rate of the plant (Capinera, 2001; Malais & Ravensberg, 2002). Prolonged aphid infestation could result in a reduction in crop yield. Together with the ability to transmit viruses (Rabasse & Steenis, 2000), the occurrence of aphids is not desired.



Figure 1.1: Aphids (*Myzus persicae*) infesting a sweet pepper flower.

In horticulture there is an increasing interest in controlling aphids biologically as the application of effective insecticides gets more and more restrained (van Lenteren, 2000). On the one hand this is due to the negative impact on the environment: effective chemicals against aphids, such as Imidacloprid, contaminate the surface water considerably (Messelink, personal communication). On the other hand supermarket chains sharpen the maximum residue levels (MRL), forcing the growers to use other control methods. Moreover, the increasing use of biological control against other pests necessitates also compatible control measures against this particular pest species (Rabasse & Steenis, 2000). Natural enemies are commonly and successfully used to control pest populations in greenhouses (van Lenteren, 2000). For the control of aphids a diverse range of commercially available natural enemies exists such as the parasitic wasps *Aphidius ervi*, *Aphidius colemani*, *Aphelinus abdominalis* and predators as *Aphidoletes aphidimyza*, *Adalia bipunctata*, *Chrysoperla carnea* and

Episyrphus balteatus (Malais & Ravensberg, 2002; Enkegaard & Brødsgaard, 2006). However, it is found that in certain situations natural enemies of aphids can't establish easily in the crop. It is of importance to know what causes this uneasy establishment to be able to effectively control this pest species.

1.2 Problem description and objectives

1.2.1 Pest species: *Myzus persicae*

One of the commonly occurring aphid species in greenhouse vegetables is the green peach aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Myzus persicae* is considered to be the most important vector of plant viruses throughout the World and around 100 viruses are known to be transmitted by this species (Capinera, 2001). Same as other aphid species, it is able to increase rapidly in population size. This is due to their simplified structure, which enables them to perform best in feeding and reproduction, with most of the nutrients directed to reproduction (Rabasse & Steenis, 2000). Depending on the host plant, presence of natural enemies and temperature (Yano, 2006) it takes *Myzus persicae* on average 10-12 days to complete a generation with 20 generations a year. It has a total longevity of 41 days and a main fecundity of 75 offspring. (Capinera, 2001)

Where suitable host plants cannot persist, *Myzus persicae* adults mate and deposit eggs on *Prunus* spp. It overwinters in the egg stage. In spring, the eggs hatch as soon as the plant breaks dormancy and begins to grow. The developed nymphs are in general yellowish/greenish but can vary in phenotype, with nymphs being more red or darker green in color, differing in reproduction rate and pesticide resistance (Gillespie, *In press*). The nymphs develop quickly, greatly resembling viviparous adults (nymph-producing, parthenogenetic) (Capinera, 2001). The aphids feed on flowers, young foliage and stems as the plant transports most of its nutrients to these newly formed plant regions (Emden et al., 1969). This can ultimately lead to a growth reduction of the plant. After several generations alate (i.e. winged) dispersants develop which are able to exploit a new area. *Myzus persicae* is able to colonize nearly all plants available (Capinera, 2001). After invading a crop, numbers increase exponentially and a new population has established. The ability to increase in such rates makes the control of aphids as pest species rather difficult.

1.2.2 Specialist predator: *Aphidoletes aphidimyza*

The most widespread and polyphagous predator used in aphid control and produced on wide scale is *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) (Yano, 2006). It can attack most of the common aphid species. The adults are fragile, short-living and small (2.5

mm long) insects and feed on honeydew. Mainly at night, adults mate and oviposition occurs



Figure 1.2.2: Larvae of *Aphidoletes aphidimyza* feeding on an aphid.

(Markkula et al., 1979). The adults are preferred as being very mobile and having good searching abilities to aphid colonies: it is found that gallmidge adults are able to locate a single infested *Brassica* plant among 75 non-infested plants in a greenhouse (El-Titik, 1973). These predators establish rapidly and oviposit according to aphid density, generating a large impact on aphid control (Choi et al., 2004). Single or

in clusters, eggs are laid on foliage within or close to aphid colonies (Choi et al., 2004) and hatch within 2.5 days (at 20°C; Malais & Ravensberg, 2002). This is crucial as *A. aphidimyza* larvae starve to death if they emerge more than 63 mm from the food (Choi et al., 2004). The gallmidge is predatory in the larval stage and the larvae are considered to feed exclusively on aphids (Christensen et al., 2002; fig 1.2.2). It pierces the herbivores body with its mandibles, injecting a paralyzing toxin, and then sucking up the preys' content (Lucas & Brodeur, 2001). The larval voracity is limited and gallmidges can develop on a relatively low number of aphids in contrast with other predators. When the prey is present in abundance, the gallmidge larvae can kill more prey than they are able to feed on (Markkula et al., 1979).

As in a soilless culture of sweet pepper and eggplants pupation is difficult (gallmidges mostly pupate in the top soil layer; Markkula et al., 1979), the release must often be repeated after 2 to 4 weeks. As these predators, in contrast to parasitoids, are quite expensive and die out when aphid densities are low, it is of importance that this predator can function well when released.

1.2.3 Intraguild predation

As biological growers have to deal with more than one pest in most cases, they release a series of beneficials (Christensen et al., 2002). Some are specialists and feed only on a certain pest insect. Others are generalists and can consume a broad range of prey (Rosenheim, 1995). This group of predators is appreciated as they can simultaneously attack different unrelated prey species and can have an impact on several pest populations (Albajes & Alomar, 2000; Montserrat, 2000). However, the possibility exists that these polyphagous control agents feed on more species encountered, pests or beneficial. In case of the latter, one speaks of intraguild predation (IGP) which can be defined as two species that share a host or prey also engage in a trophic interaction with each other (parasitism or predation) (Polis et al., 1989; Polis & Holt, 1992; Hindayana et al., 2001; Venzon et al., 2001; Yano, 2006). Theory predicts an unstable equilibrium in this simple three species model (Holt & Polis, 1997). Intraguild predation could namely be intense, resulting in high mortality of the victim (the other predator; the IG

prey) while the total mortality imposed upon the shared prey population (the target pest) is minimal (Polis et al., 1989). Under these conditions IGP can lead to a breakdown of biological control (Rosenheim, 1995). This assumption led to a tremendous increase of interests in insect behaviour and intraguild predation within agricultural and horticultural communities.

Reviews of Rosenheim et al. (1995), Lucas (2005) and Janssen et al. (2006) listed a growing number of empirical studies demonstrating the common occurrence of IGP within communities of biological control agents. Although theory opposes the concerns on biocontrol, the widespread occurrence of IGP assumes the presence of a stable equilibrium. A likely explanation for the contradiction between theory and reality is the complexity within communities. Different factors are found to influence predator interactions, such as prey specificity and relative body size of the predators (Polis et al., 1989), in which the larger predator exploits the smaller one (Hindayana et al., 2001). Additionally, the guild, foraging habitat (Janssen et al., 2007), mobility and aggressiveness of the predators (Lucas et al., 1998^b) and the availability of extraguild prey (Hindayana et al., 2001; Daugherty et al., 2007) can influence the occurrence of IGP.

Although the reviews assume a minimal effect of IGP on the ultimate biological control, one must say that in most cases a general comprehensive conclusion is made, based on a variety of studies in which different predators were investigated (Janssen et al., 2006). It is presumable that the occurrence of IGP is dependent on the particular species concerned. Accordingly, the effect of IGP between aphidophagous predators on the biological control of aphids must not be neglected before empirical evidence shows the likelihood of disturbing aphid control by IGP. Studies attesting the presence of intraguild predation between aphidophagous control agents used in the biological production of greenhouse vegetables, are listed in table 1.2.1. Although already an extensive list exists, only a few studies investigated the effect of IGP on pest control. Furthermore, it shows a high number of IGP studies on ladybeetles (Coccinellidae; 14 related studies), but available literature of IGP on other important natural enemies of aphids is significantly smaller. Especially concerning *Aphidoletes aphidimyza* (5 studies), which is known to be the most widely used aphid predator in greenhouses. The immature stages of the gallmidge are very vulnerable to predation (Lucas et al., 1998^b; van Schelt, 2000) as being sessile, slow moving and highly specialized, not able to use its mouth parts effectively toward attacking other predatory organisms (Lucas et al., 1998^b). It is likely that other control agents may prey upon this specialist natural enemy when present together (IGP), affecting aphid control.

Table 1.2.1: Summary of studies on intraguild predation among aphidophagous control agents used commercially in greenhouses (Enkegaard & Brødsgaard, 2006; Yano, 2006). The experiment was either a laboratory (L), field (F) or field cage study (FC). Ni = Not investigated. V = Victim, E = Exploiter.

Reference	Type	Effect on biological control	Interacting Species	Family
Alhmedi et al (2007)	F	Ni	<i>Episyrphus balteatus</i> (V,E) <i>Coccinella septempunctata</i> (V,E)	Syrphidae Coccinellidae
Bilu et al. (2006)	FC	Ni	<i>Aphidius colemani</i> (V) <i>Coccinella undecimpunctata</i> (E)	Aphidiinae Coccinellidae
Bilu & Coll (2007)	FC, L	0	<i>Aphidius colemani</i> (V) <i>Coccinella undecimpunctata</i> (E)	Aphidiidae Coccinellidae
Brødsgaard & Enkegaard (1995)	FC	0	<i>Aphidoletes aphidimyza</i> (V) <i>Orius majusculus</i> (E) <i>Phytoseiulus persimilis</i> (V)	Cecidomyiidae Anthocoridae Phytoseiidae
Chacon et al. (2007)	F	0	<i>Chrysoperla carnea</i> (V) <i>Harmonia axyridis</i> (E)	Chrysopidae Coccinellidae
Christensen et al (2002)	L	Ni	<i>Aphidoletes aphidimyza</i> (V) <i>Orius majusculus</i> (E)	Cecidomyiidae Anthocoridae
Enkegaard et al. (2005)*			<i>Aphidius colemani</i> (V) <i>Aphidoletes aphidimyza</i> (E)	Aphidiinae Cecidomyiidae
Fréchette, B. et al (2007)	L	Ni	<i>Episyrphus balteatus</i> (E,V) <i>Macrolophus caliginosus</i> (mainly E,V) <i>Sphaerophoria rueppellii</i> (E,V) <i>Sphaerophoria scripta</i> (E,V)	Syrphidae Miridae Syrphidae
Gardiner & Landis (2007)	FC	0	<i>Aphidoletes aphidimyza</i> (V) <i>Chrysoperla carnea</i> (V) <i>Harmonia axyridis</i> (E)	Cecidomyiidae Chrysopidae Coccinellidae
Hemptinne et al (2000)	L	Ni	<i>Adalia bipunctata</i> (V,E) <i>Coccinella septempunctata</i> (V,E)	Coccinellidae
Hindayana et al. (2001)	L	Ni	<i>Aphidoletes aphidimyza</i> (V) <i>Coccinella septempunctata</i> (V,E) <i>Chrysoperla carnea</i> (V,E), <i>Episyrphus balteatus</i> (V,E)	Cecidomyiidae Coccinellidae Chrysopidae Syrphidae
Hironori & Katsuhiko (1997)	F	Ni	<i>Coccinella septempunctata</i> , (V,E) <i>Harmonia axyridis</i> (V,E)	Coccinellidae
Jazzar et al (2008)	L	Ni	<i>Aphelinus abdominalis</i> (V) <i>Chrysoperla carnea</i> (E)	Aphidiinae Chrysopidae
Lucas et al. (1998)	L	Ni	<i>Aphidoletes aphidimyza</i> (V) <i>Chrysoperla rufilabris</i> (E) <i>Coleomegilla maculata</i> (E)	Cecidomyiidae, Chrysopidae Coccinellidae
Meyling et al. (2004)	L	Ni	<i>Aphidius colemani</i> (V) <i>Anthocoris nemorum</i> (E)	Aphidiinae
Nakashima et al. (2004)	L	Ni	<i>Aphidius ervi</i> (V) <i>Coccinella septempunctata</i> (E)	Aphidiinae Coccinellidae
Nakashima & Senoo (2003)	L	Ni	<i>Aphidius ervi</i> (V) <i>Coccinella septempunctata</i> (E)	Aphidiinae Coccinellidae
Nakashima et al. (2006)	L	Ni	<i>Adalia bipunctata</i> (E) <i>Aphidius ervi</i> (V), <i>Coccinella septempunctata</i> (V) <i>Praon volucre</i>	Coccinellidae Aphidiinae Coccinellidae Aphidiinae
Phoofolo & Obrycki (1998)	L	-	<i>Chrysoperla carnea</i> (V) <i>Coleomegilla maculata</i> (E) <i>Coccinella septempunctata</i> <i>Harmonia axyridis</i> (E)	Chrysopidae Coccinellidae
Pinada et al. (2007)	FC	+	<i>Aphidius colemani</i> (V) <i>Episyrphus balteatus</i> (E)	Aphidiidae Syrphidae
Rosenheim et al. (1993)	L	-	<i>Chrysoperla carnea</i> (V) <i>Nabis spp.</i> (E) <i>Zelus renardii</i> (E)	Chrysopidae Nabidae Reduviidae
Santi & Maini (2006)	L	Ni	<i>Adalia bipunctata</i> (V) <i>Chrysoperla carnea</i> (E) <i>Harmonia axyridis</i> (V) <i>Orius leavigatus</i> (E)	Coccinellidae Chrysopidae Coccinellidae Miridae

* Not able to obtain this article; described by other studies.

1.3 Aim of the study

Orius spp. (Hemiptera: Anthocoridae) and *Macrolophus caliginosus* Wagner (Heteroptera: Miridae) are widely used in greenhouses as control agents. The anthocorid bugs are particularly marketed as predators of thrips (Ito, 2007) and the mirid bug is mainly released to attack whitefly (Bonato et al., 2006). Although used for specific purposes, both species are widely known as being polyphagous, preying upon a variety of pest insects as thrips, whitefly, aphids, mites and other soft bodied arthropods (Albajes & Alomar, 2000). It is likely that these generalist control agents prey upon other beneficials such as *Aphidoletes aphidimyza*, probably affecting the biological control of this particular predator.

The main objective of this study is to find out if predatory bugs (i.e. *Macrolophus caliginosus*, *Orius laevigatus* and *Orius majusculus*) affect aphid control by *Aphidoletes aphidimyza*. Three research questions are put forward: (i) to what extent do these predatory bugs affect biological control of aphids by *Aphidoletes*, (ii) what is the role of alternative prey in these IGP interactions and (iii) does prey preference or predator switching affect IGP? It is hypothesised that the effective control of aphids by *A. aphidimyza* is disrupted as the generalist predators interact with *A. aphidimyza* by eating the gallmidges' eggs and larvae. Alternative food for the predatory bug (i.e. whitefly for *M. caliginosus* and thrips for the *Orius* spp.) is thought of reducing the occurrence of IGP on gallmidges, as the predator will prey upon its main and preferred food source.

2 Material and methods

2.1 Rearing and collecting of prey and predators

Of all experimental insects, i.e. (i) the specialist predator *Aphidoletes aphidimyza*, (ii) the generalist predatory bugs *Macrolophus caliginosus*, *Orius majusculus* and *Orius laevigatus*, and (iii) the pest species *Myzus persicae*, *Frankliniella occidentalis* and *Trialeurodes vaporariorum*, an attempt was made to obtain the insects from own rearing. As the rearing of the predatory bugs failed, insects from production companies were used.

Myzus persicae (Sulzer). Aphids were obtained from a rearing in a walk-in greenhouse with infested sweet pepper plants. In all experiments a certain number of mixed instars were collected using a fine paintbrush.

Frankliniella occidentalis (Pergande). Thrips adults were collected from a rearing on chrysanthemum, making use of an aspirator. To obtain second instar thrips larvae, thrips adults were placed in a glassbottle with fine gauze in lid, containing a filterpaper, pollen (to enhance egg laying; Malais & Ravensberg, 2002) and beans as oviposition substrate and supply of moisture. After two days, beans with eggs were taken out and placed in a new glassbottle to obtain larvae of similar age. Climatic conditions: 25°C, L:D 16:8 h, 70% RH.



Figure 2.1.1: Rearing of anthocorid bugs (above) and thrips (below) on beans in a climate cabinet.

Trialeurodes vaporariorum (Westwood). Whitefly adults were obtained from *Koppert BV* (the Netherlands) and reared on cucumber plants. Adults were collected in pipette-tips using an aspirator.

Orius laevigatus and *Orius majusculus*. Fresh adults were obtained from *Koppert BV* (The Netherlands) and *Biobest N.V.* (Belgium), respectively. An attempt was made to obtain anthocorid bugs from own rearing according to a method described by Meiracker (Meiracker, 1999)¹. When the rearing was set up, the delivered predators were of bad quality and rearing failed. When the experiments started, the quality of the delivered predators was better and readily used for the experiments.

¹ Predators were released in glassbottles (11 x 11 x 20 cm) with fine gauze in lid and placed at 25°C, L:D 16:8 h, 70% RH. Twice per week, predators were fed eggs of the flour moth, *Ephestia kuehniella* (Zeller), and provided with beans as oviposition substrate and supply of moisture. Beans with predator eggs were collected after 3 days and placed in a new glassbottle with flour moth eggs. A piece of filterpaper was laid on the bottom to take up moisture and a crumpled tissue was added to provide hiding places for the juveniles, reducing cannibalism.

Macrolophus caliginosus. Adults were obtained from *Biobest BV* (The Netherlands). For the small cage experiment, *M. caliginosus* was reared on tomato plants in net covered cages infested with whitefly. Ephestia eggs were added each week as additional food source.

Aphidoletes aphidimyza. Pupa were obtained from *Koppert BV* (The Netherlands). For the lab experiments, *A. aphidimyza* larvae were collected from a walk-in greenhouse compartment with aphid infested sweet pepper plants. Gallmidge pupa were placed in the greenhouse 2 weeks before a prey preference experiment (by placing pupa in petridishes with moisture vermiculite) in order to have access to 3rd instar larvae at start of the prey preference test.

2.2 Experimental design

Greenhouse experiments were performed to investigate the effect of the predatory bugs *Macrolophus caliginosus* (exp A) and *Orius laevigatus* and *Orius majusculus* (exp B) on aphid control by gallmidges. A laboratory experiment (C) with the anthocorid bugs was done to examine if the predatory bugs preyed upon *A. aphidimyza* larvae (IGP) and if they would have a preference when both *A. aphidimyza* larvae and their main food source were present (i.e. thrips). The experiments were carried out at *Wageningen UR Greenhouse Horticulture*, Bleiswijk (The Netherlands).

2.2.1 Effect of *M. caliginosus* on aphid control by gallmidges (A).

Table 2.2.1: Description of the treatments from the greenhouse (Grh) and laboratory experiments (Lab). In the lab experiment (C) only the two Orius bugs were observed on prey preference. Five replicates of each treatment in the greenhouse experiments, 10 replicates in the laboratory experiment. Due to a limited number of plants in experiment A, only 4 treatments were incorporated.

Exp	Lab/Grh	Treatments	Plant
A	Grh	a <i>Myzus persicae</i>	Eggplant
		b <i>Myzus persicae</i> + <i>A. aphidimyza</i>	
		c <i>Myzus persicae</i> + <i>A. aphidimyza</i> + <i>M. caliginosus</i>	
		d <i>Myzus persicae</i> + <i>A. aphidimyza</i> + whitefly + <i>M. caliginosus</i>	
B	Grh	a <i>Myzus persicae</i>	Sweet pepper
		b <i>Myzus persicae</i> + <i>A. aphidimyza</i>	
		c <i>Myzus persicae</i> + <i>A. aphidimyza</i> + <i>O. majusculus</i>	
		d <i>Myzus persicae</i> + <i>A. aphidimyza</i> + <i>O. majusculus</i> + thrips	
		e <i>Myzus persicae</i> + <i>O. majusculus</i> + thrips	
		f <i>Myzus persicae</i> + <i>A. aphidimyza</i> + <i>O. laevigatus</i>	
		g <i>Myzus persicae</i> + <i>A. aphidimyza</i> + <i>O. laevigatus</i> + thrips	
		h <i>Myzus persicae</i> + <i>O. laevigatus</i> + thrips	
C	Lab	a <i>M. persicae</i> + <i>A. aphidimyza</i> larvae + <i>O. laevigatus</i>	
		b <i>M. persicae</i> + <i>A. aphidimyza</i> larvae + thrips larvae + <i>O. laevigatus</i>	
		c <i>M. persicae</i> + <i>A. aphidimyza</i> larvae + <i>O. majusculus</i>	
		d <i>M. persicae</i> + <i>A. aphidimyza</i> larvae + thrips larvae + <i>O. majusculus</i>	

To find out (i) the effect of *M. caliginosus* adults on aphid control by gallmidges and (ii) if alternative food (i.e. whitefly) for the predatory bug would enhance or diminish this effect, a plant experiment was performed. Twenty eggplants (*Solanum esculentum*, 30-40 cm, ± 12 leaves, 1 flowerbud), were placed in cages (30 cm \varnothing , 40 cm h; top and side-openings covered with fine gauze) in a greenhouse compartment (shown in picture 2.2.1). Due to the large size of the plants the cages were lifted using plastic pots (16 cm at start, 20 cm halfway the experiment) and a waterproof hardboard in which a hole (10.5 cm) was bored to be able to press the substrate of the plant through. The opening was closed using plastic, tape and parafilm (between the stem and plastic). Each plant served as one replicate with five replicates per treatment (table 2.2.1). Possible direct or indirect (plant-mediated or predator-mediated) interactions between aphids and whiteflies were not investigated, due to the limited number of available plants. Temperatures in the greenhouse compartment fluctuated with an average of 23.4 °C (figure 2.2.2). Plants were watered by hand as little as possible to minimize plant transpiration, which could otherwise cause condensation on the inside of the cages resulting in death of gallmidge adults. The relative humidity of the greenhouse was kept as low as possible and was on average 64%. In the individual cages this value was higher.



Figure 2.2.1: Plants were covered by cages to be able to introduce insects to a single plant.

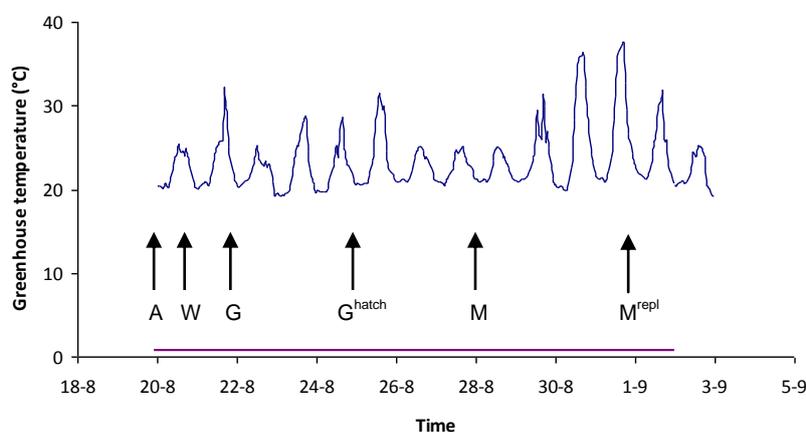


Figure 2.2.2: Greenhouse temperature during the experiment and time of insect release. Average temperature of 23.4°C (maximum of 37.7°C at daytime, minimum of 20.0°C at night). The purple line indicates the duration of the experiment (i.e. 14 days). Arrows indicate introduction date of insects: 20 aphids (A), 10 gallmidge pupa (G), 20 Whitefly adults (W) and/or 10 *Macrolophus caliginosus* adults (M) per individual plant. (G^{hatch} = when 1st gallmidges hatched; M^{repl} = dead predatory bugs replaced by new ones). The experiment was stopped when in one of the cages almost all aphids were eaten.

As the plants were lightly infested by thrips, the percentage of thrips infestation was recorded prior to the experiment (0.375 thrips leaf⁻¹). The insects were placed on the plants at different time points (shown in figure 2.2.2), starting with the pest insects and followed by the

predators. In this way the pest insects were able to increase in population size before predators were present. To estimate the number of hatched female/male gallmidges a pot of 100 pupa was placed in the greenhouse and each day checked on gallmidge emerge: $\pm 71\%$ of which 44% male and 56% female. The predatory bug *M. caliginosus* was added when all gallmidges were hatched. The insects (except for the aphids which were placed on the plant at start) were added by lifting up the cages slightly and placing the petridish or pipettetip with insects on the bottom of the cage. Nine days after the first emerge of gallmidge adults the experiment was stopped. At that time the *A. aphidimyza* larvae had eaten most of the aphids in one of the cages and in the control cages fungi started to develop. The cages were lifted carefully and the number of living *M. caliginosus* adults was counted by checking each plant for 2 minutes. It was not able to count the exact number as some flew away when lifting the cage. The counting of the predatory bugs was done to ensure that there were still living predatory bugs present at time of finishing. As almost all insects were located in the top of the plant (the part above the first flower; $\pm 10-14$ small/large leaves), only this part was taken, put in plastic bag and brought to the laboratory. Total numbers of aphids (alive) and gallmidges (larvae, eggs; alive, dead, eaten) were counted. Due to a high number of whitefly (all stages), this pest species was recorded as being present in abundance.

To support final observations, a small additional experiment was done to see if *Macrolophus caliginosus* would be attracted to the top of the plant in the presence of whitefly (change in foraging habitat, enhancing encounter rates with gallmidges). 10 predatory bugs were released on an aphid infested and an aphid + whitefly infested plant and left for 48 hours, after which the number of *Macrolophus* on the top and lower part of the plant was recorded (top defined as the part of the plant above the second flowers; the plants had 5 flowers in 3 levels (1,2,2)).

2.2.2 Effect of *Orius* spp. on aphid control by gallmidges (B).

Similar greenhouse experiment as the previous, but in this case the effect of the predators *Orius majusculus* and *Orius laevigatus* was investigated (table 2.2.1; fig. 2.2.3). An extra



Figure 2.2.3: Set up of sweet pepper plants in cages, in the greenhouse compartment. 40 cages were placed (8 treatments, of each 5 replicates).

treatment with ‘aphids, the predatory bug and thrips’ was incorporated to observe the effect of the predatory bug on aphids. The experiment was performed using sweet pepper plants (*Capsicum annuum*; variety *Ferrari*; ± 24 cm h, 15-17 leaves, 3 flowerbuds).

As alternative food, the thrips species *Frankliniella occidentalis* (Pergande) was

supplied. Five replicates per treatment. As at start a single thrips was noticed 4 random leaves per plant (160 in total) were checked on thrips infestation (0.01875 thrips leaf⁻¹). If a thrips larvae or adult was found it was killed and the infested plant was used in one of the treatments with thrips, just in case the plants contained any thrips eggs. Some of the released aphids were parasitized, causing a slow population development (figure 2.2.4). To prevent the presence of



Figure 2.2.4:
Mummified
aphid.

parasitoids, the cages were taken away every two days and each plant was checked for 2 minutes on mummified, black or suspicious looking aphids which were replaced with fresh ones. Due to lifting of the cages more thrips adults were released in the cages twice (2, 11 and 21 days after aphid release; 20, 20 and 40 female adults respectively; figure 2.2.5). Lifting of the cages was stopped when the gallmidge adults emerged. Out of the 10 gallmidge pupa added, 8 to 9 (54% male, 46% female) emerged in each of the cages with gallmidges. The experiment was stopped 13 days after emerging of the gallmidges, when most of the aphids were eaten in one treatment and fungi started to develop. This was considerably later than in the previous experiment, probably caused by the reduced development rate of the gallmidges. The large difference in temperature between the experiments could likely explain the delayed activity of the gallmidges. As the insects were found on all parts of the plant, the whole plant was checked on insect numbers making a distinction between the top and lower part of the plant. In 7 out of the 8 treatments parasitisation was found in one of the replicates. As the intensity of parasitisation varied the samples were not used for the analysis, thus resulting in 4 replicates per treatment. Temperature was controlled to obtain a minimum of 20°C (figure 2.2.5). During daytime, when light levels outside felt below 100 J/cm, an assimilation light was turned on to stimulate the development of the insects. It was switched off halfway the experiment in order to exclude any influence on the behaviour of the gallmidge adults.

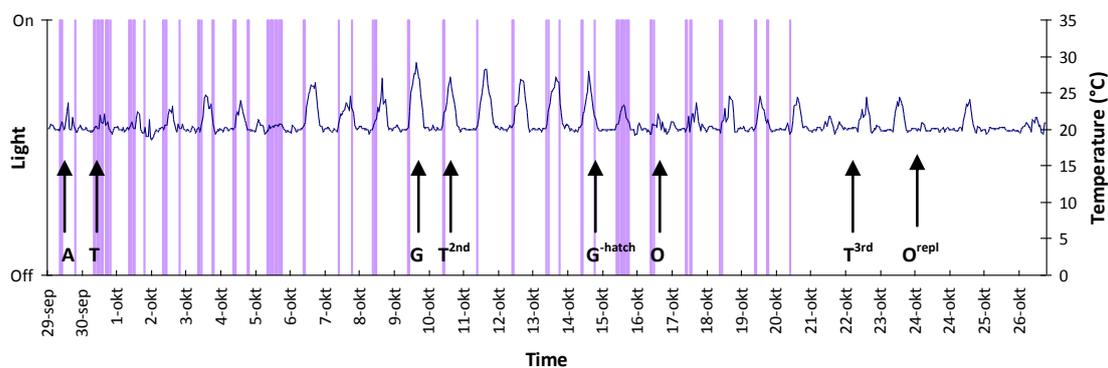


Figure 2.2.5: Greenhouse temperature (blue line), additional light supply (purple bars) and time of insect release during the experiment. Average temperature was 20.9°C (maximum of 29.2°C during daytime, minimum of 18.2°C at night). The light was switched off halfway the experiment. The total duration of the experiment was 30 days (11 days from when all insects were present). Arrows indicate introduction date of insects: 20 aphids (A), 10 gallmidge pupa (G), 20/20/40 thrips adults respectively (T) and 10 *Orius majusculus* or *laevigatus* adults (O) per individual plant. (G^{hatch} = when 1st gallmidges hatched. O^{repl} = dead predatory bugs replaced by new ones). The experiment was stopped when in one of the cages almost all aphids were eaten/fungi appeared.

2.2.3 Prey preference of the anthocorid bugs.

To ground the behaviour of the predatory bugs in the greenhouse, it was investigated in small laboratory experiments if *O. laevigatus* and *O. majusculus* adults would consume *A. aphidimyza* (occurrence of IGP) when it is supplied together with aphids (to simulate reality in which both species also occur). Furthermore, it was determined if the availability of the alternate prey would affect the tendency of the predatory bugs to kill *A. aphidimyza* larvae (reducing IGP). The prey preference of the mirid bug was not investigated as time was limited and not enough *A. aphidimyza* larvae were available.

Table 2.2.2: Methods tested on suitability for prey preference laboratory tests.

Method	Leaf part	Experimental area
1.	Single leaf	a) In wet oasis; placed in glassbottle with fine gauze in lid.
2.	Leaf punch	a) In wet filter paper or agar; b) Petridish (1.5 cm height) or plastic cup (3 cm height); c) With/without ventilation.

Different experimental areas were tested to find the most suitable method (shown in table 2.2.2 and figure 2.2.6). The method in which a leaf punch is placed in a petridish (with/without wet filter paper underneath; used often in similar experiments) was not usable. Without wet filter paper the sweet pepper leaf did not keep fresh for 24h and with wet filter paper the aphids and Orius died when getting in contact. Additionally, the aphids, gallmidges and thrips were able to go underneath the leaf although the Orius bug was not able to do. Finally, the leaf transpired highly, causing condensation on the lid to which the Orius stuck and consequently died. The second option with a single leaf placed in wet oasis in a glassbottle with fine gauze in lid was promising but not useable either. Gallmidges did not stay on the leaf and were found back on the inside of the pot or were able to crawl into the oasis, even when it was closely frapped with parafilm. Data from this experiment were not used. As a result plastic cups (6.5 Ø diameter, 3 cm h) were used in which easily a ventilation hole with fine gauze in lid could be made (2 x 2 cm). A 0.4 cm wateragar layer² was used to



Figure 2.2.6: Methods tested. (a) Petridish: condense in lid, (b) glassbottle with leaf in oasis: insects left the leaf, (c) plastic cup with ventilation in lid and agar layer. Method C was used.

² Preparation of 1 L wateragar: A mixture of 15gr of agar (Agar Technical Solidifying agent, Difco) to which 1 L demiwater was added, was placed in autoclave at 120°C (15 psi pressure) for 20 minutes. The mixture was cooled down till 60°C to be able to pour it into the plastic cups.

keep the leaf punch fresh for 2 days. The thickness was chosen as a thinner layer easily got loose from the sides of the plastic cup, resulting in gallmidge larvae and thrips crawling underneath the agar. The leaf was pressed into the agar on the sides to minimize insects hiding underneath the leaf. The lid was placed upside down as insects could crawl between the lid and cup. Two layers of parafilm were used to close the cup.

To obtain a high number of aphids on a small piece of leaf, aphids were placed on clean sweet pepper plants and left for 2 weeks to increase in numbers. Leaf punches with aphids were pressed one day prior to the experiment; placed in the cup with agar; more aphids were added (to obtain on each leaf 100 aphids) and left for 1 day in the climate chamber at 25°C (L:D 16:8 h, 70% RH) to increase quickly in numbers. In case of *Orius laevigatus* the sweet pepper leaf punches (5 cm diameter) contained ≤ 140 aphids at start. Due to the low number of leaves with aphids, radish leaf punches containing ≤ 180 aphids were used for the prey preference test with *Orius majusculus*. As prey, 20 3rd instar gallmidge larvae were offered to a single predatory bug with or without 2nd instar *Frankliniella occidentalis* larvae. The number of prey was based on the maximum amount *Orius spp.* can consume within 24h (van Lenteren et al., 1997).

Prior to experimentation, predators were starved for 24h by placing them individually in petridishes with wet filter paper. At the start of the experiment a single predator was added to the experimental area and tested on feeding behaviour for a 24-h period in a climate cabinet at 25°C, L:D 16:8 h, 70% RH. 10 replicates of each treatment (choice/ no choice; table 2.2.2). At the end the predator was removed and the number of aphids (dead) and *A. aphidimyza* and thrips larvae (eaten/uneaten) were counted.

2.3 Statistical analysis

Greenhouse experiment. Data of greenhouse experiments were subjected to Analysis of Variance (ANOVA) using Genstat. Significance was defined as $P \leq 0.05$. In order to stabilise variance, the number of aphids were log transformed before the analysis.

Prey preference experiment. As the number of gallmidges eaten by the predatory bug were low (1-3) in both treatments, no preference index could be calculated. The predation by the predatory bugs in the two treatments was analysed using proportions of total number of prey killed (number killed/number offered). Differences between the numbers of gallmidges killed were subjected to Analysis of Variance to check if still a significant difference could be observed.

3 Results



3.1 Effect of *Macrolophus caliginosus* on aphid control by gallmidges

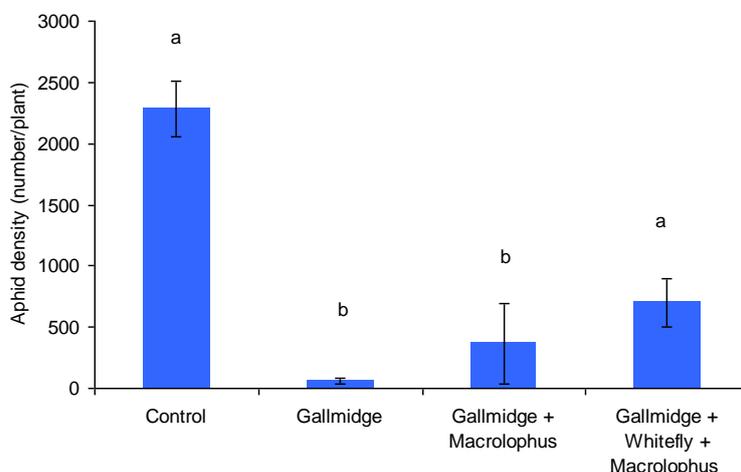


Figure 3.1: The effect of *Macrolophus caliginosus* on aphid control by the gallmidge *Aphidoletes aphidimyza*, in absence/presence of alternative food for the mirid bug (the whitefly *Trialeurodes vaporariorum*). Shown are average aphid densities (+SEM), each bar being the average of 5 replicates (5 plants). The control treatment consisted out of aphid infested plants. $P < 0.001$.

The number of aphids reduced drastically in the presence of gallmidges ($P < 0.001$), from an average of 2300 to 60 aphids per plant (figure 3.1). In the accompanied presence of *Macrolophus* the number of aphids seemed to increase slightly although no significant difference was observed. When alternative food for the predatory bug was present in abundance, the aphid density was significantly higher in comparison to the treatments without whitefly ($P < 0.001$).

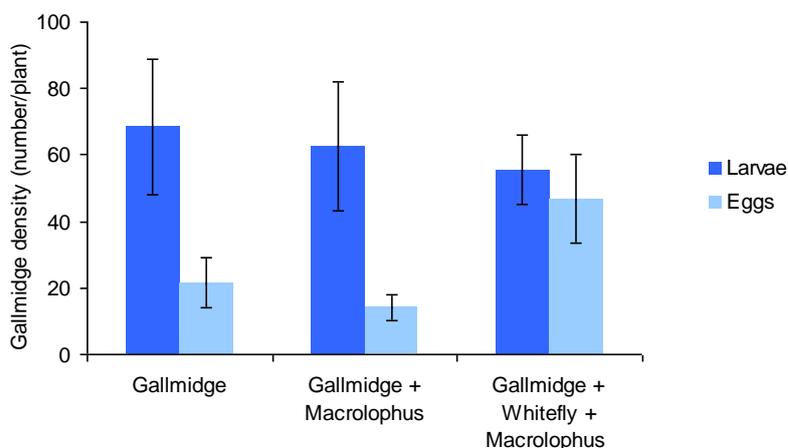


Figure 3.2: The effect of *Macrolophus caliginosus* on the gallmidge *Aphidoletes aphidimyza*, in absence/presence of alternative food for the mirid bug (the whitefly *Trialeurodes vaporariorum*). Shown are average gallmidge larvae and egg densities (+SEM), each bar being the average of 5 replicates (5 plants). The control treatment consisted out of aphid infested plants. No significant differences were found between treatments (larvae: $P = 0.87$, l.s.d. 53.6) (eggs: $P = 0.06$, l.s.d. 28.2).

Although the number of gallmidge larvae seems to be lower in the treatments with *Macrolophus* and *Macrolophus* + whitefly, no significant differences were found. Also no significant differences were found between the treatments in number of gallmidge eggs ($P = 0.06$). Remains of attacked and dead gallmidge larvae were found in treatments with *Macrolophus*, although numbers were low (± 1 per plant).

3.2 Effect of anthocorid bugs on aphid control by gallmidges



3.2.1 Prey preference

Table 3.1: Predation, expressed as numbers eaten in 24 hours, by adults of the anthocorid bugs *Orius majusculus* and *Orius laevigatus* when exposed to 20 3rd instar gallmidge larvae solely (no choice) or together with 20 2nd instar thrips larvae (choice). Aphids were in both treatments present in abundance (≥ 140 on a sweet pepper leaf for *O. laevigatus*, ≥ 180 on Radish leaf for *O. majusculus*) to simulate reality (gallmidges are only introduced and present when aphids are in the greenhouse). Values are the average of 10 replicates. The predatory bug was starved for 24h prior to the experiment.

Predator	Prey	Predation rate (Number eaten day ⁻¹)		P	l.s.d.
		No choice	Choice		
<i>Orius majusculus</i>	<i>A. aphidimyza</i>	1.0	0.9	NS	1.231
	Thrips	*	8.3		
	Aphids*	73.5	72	NS	9.370
<i>Orius laevigatus</i>	<i>A. aphidimyza</i>	0.7	1.0	NS	0.942
	Thrips	*	2.1		
	Aphids*	57.7	64.5	NS	14.61

Note: * Eaten both by the gallmidge larvae and *Orius* bugs.

Both adults of *Orius majusculus* and *Orius laevigatus* preyed upon *A. aphidimyza* larvae, although the numbers were low (0.7 – 1.0 larvae for *O. laevigatus* and 1.0 – 0.9 for *Orius majusculus* within 24h, in the choice and no choice treatments respectively). Addition of thrips larvae did not lead to any difference in gallmidge consumption. The number of thrips eaten by *Orius laevigatus* was low, but *Orius majusculus* consumed 41 percent of the thrips present (8.3 larvae out of the 20 introduced). In both cases the number of aphids eaten did not differ between the two treatments.



Figure 3.3: Three gallmidge larvae, of which two are attacked by *Orius laevigatus*.

3.2.2 Greenhouse experiment

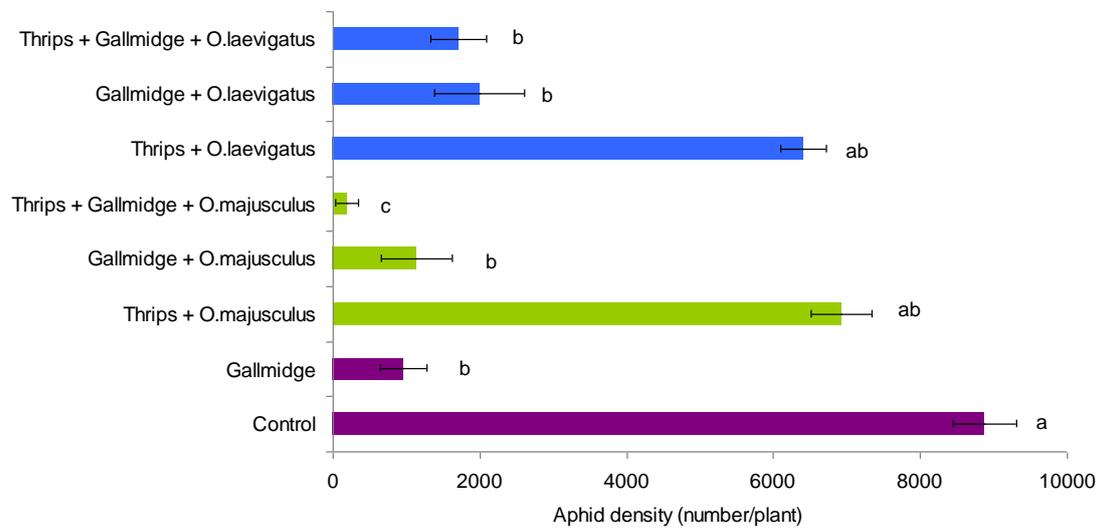


Figure 3.4: The effect of *Orius majusculus* (green bars) and *Orius laevigatus* (blue bars) on aphid control by the gallmidge *Aphidoletes aphidimyza*, in absence/presence of alternative food for the anthocorid bugs (the thrips *Frankliniella occidentalis*). Shown are average aphid densities (+SEM), each bar being the average of 4 replicates (4 plants). The control treatment consisted out of plants infested only with the aphid *Myzus persicae*. $P < 0.001$.

Just as in the greenhouse experiment with *M. caliginosus*, gallmidges reduced the aphid population also considerably on the infested sweet pepper plants (9x, fig 3.4). Addition of the Orius bugs showed a small tendency of an increase in aphid numbers, although not significant. The predatory bug itself (both Orius species) preyed upon aphids (fig. 3.5), but did not affect the aphid population significantly. However, the availability of alternative food for the predatory bug *Orius majusculus* resulted in lower numbers of aphids ($P < 0.001$; in average 5 times lower in comparison with the treatment with only gallmidges).

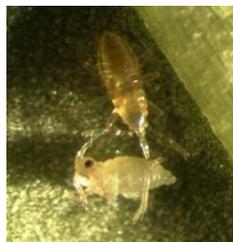


Figure 3.5: *Orius laevigatus* larvae preying upon an aphid.

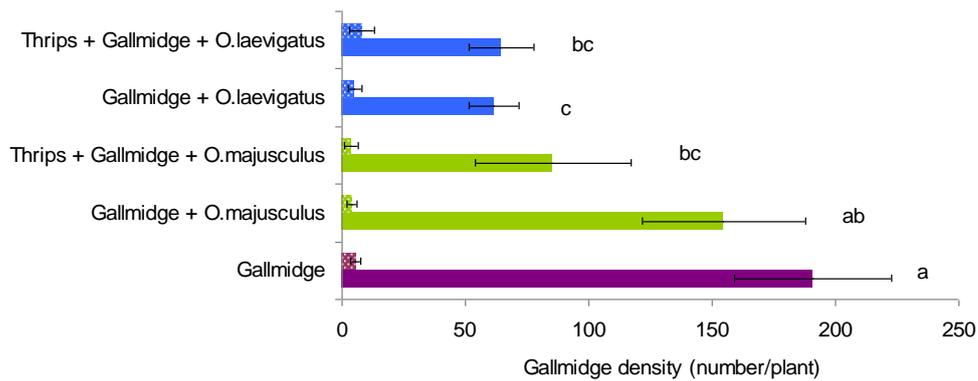


Figure 3.6: The effect of *Orius majusculus* (green) and *Orius laevigatus* (blue) on the gallmidge *Aphidoletes aphidimyza*, in absence/presence of alternative food for the anthocorid bugs (the thrips *Frankliniella occidentalis*). Shown are average gallmidge densities, larvae and eggs (dotted) (+SEM), each bar being the average of 4 replicates (4 plants). Significance was found between the density of gallmidge larvae: $P = 0.011$.

The number of gallmidges was highest when the predatory bugs were absent (fig. 3.6). In the presence of *Orius laevigatus*, the number of gallmidges reduced considerably ($P = 0.011$) independent of the availability of alternative food. Also *Orius majusculus* affected the number of gallmidges although only significant differences were found when also available food was present. In the cages with predatory bugs a significant number of attacked gallmidge larvae and eggs were found although no differences were observed between these treatments.

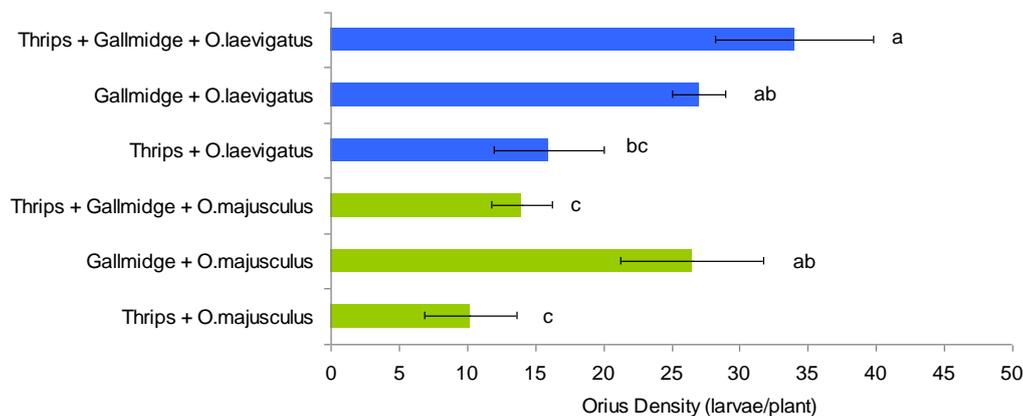


Figure 3.7: The development of *Orius majusculus* (green) and *Orius laevigatus* (blue) when released on aphid infested plants, in the presence/absence of *Aphidoletes aphidimyza* and alternative food for the predatory bug (thrips, *Frankliniella occidentalis*). Shown are average densities of Orius larvae (+SEM), each bar being the average of 4 replicates (4 plants). $P = 0.004$.

The number of Orius larvae was significantly higher when gallmidges were present (except for the treatment with thrips, gallmidges and *Orius majusculus*). Especially high numbers of *Orius laevigatus* were observed (1.7 to 2.1 times higher) in contrast to the treatment without gallmidges. (fig 3.7)

4 Discussion

In this study it was hypothesized that the generalist predatory bugs *O. laevigatus*, *O. majusculus* and *M. caliginosus* can disrupt aphid control by predating on eggs and larvae of the aphid predator *A. aphidimyza*. The obtained results confirm the generalist predation character of the anthocorid bugs and both include the gallmidge *Aphidoletes aphidimyza* in their range of prey (Brødsgaard & Enkegaard, 1995; Christensen et al., 2002). Direct predation was observed in both the prey-preference and greenhouse experiment, resulting in a lower gallmidge density.

Despite the consumption of gallmidges by the anthocorid bugs, it did not result in higher aphid densities. This result is in accordance with other empirical tests in which the simple IGP theory³ fails (Janssen et al., 2006). This suggests that intraguild predation on gallmidges must be counteracted in some way, preventing an increase in aphid numbers. It could be that the remaining gallmidge larvae compensate the loss by consuming or killing more aphids as it is known that gallmidges can kill more aphids than needed (Markkula et al., 1979). More obvious would be the explanation that the reduced predation of aphids, due to the loss of gallmidges, is counterbalanced by aphid attack of the predatory bugs. Of both *Orius* spp. it is known to prey upon aphids (Albajes & Alomar, 2000) and direct predations were also observed during the experiments (fig 3.5) supporting this assumption.

The role of thrips in this IGP interaction is not very clear. The presence of this alternative prey did not show a switching to thrips in the prey preference test and there was no effect on gallmidge population. However, the number of *Orius laevigatus* larvae was significantly higher on plants with the large prey variety, suggesting that a mixed diet enhances its development. This phenomenon was also found by another study in which predatory mites were found to have a higher growth rate when exposed to a mixed diet of whitefly and thrips (Messelink et al., 2008). Evans (2008) suggested that the mixed diet of two or more prey promote predator development by providing particularly well-balanced nutrition. So it is presumable that the IG predator receives a benefit from consuming the IG prey (i.e. the gallmidge). On the long term, this enhanced predatory bug development may increase IGP on gallmidges, negatively affecting the biological control of aphids. On the contrary, it might be beneficial for thrips control.

The development of *Orius majusculus* on the other hand, was not enhanced by the presence of thrips. However, the number of aphids and gallmidges was lower when thrips

³ The standard three-species IGP model of Polis et al. (1989) involving 3 species: a top predator (IG predator), an intermediate consumer (IG prey) and a shared sources (the pest insect), making the assumption that IGP of the top predator on the intermediate consumer causes an increase of the pest population.

were present, suggesting that the mixed and large availability of food changed the predatory bugs' behaviour. It may have caused an increased aggressiveness or a change in foraging behaviour, attacking more species encountered. This phenomenon is well documented and described by Prasad & Snyder (2006) as 'trait-mediated' indirect interactions (TMII), occurring when one species (i.e. the alternative prey thrips) modifies the interaction between a pair of species by changing the behaviour, but not density, of an intermediary species (i.e. *Orius majusculus*) (Prasad & Snyder, 2006). It is interesting to investigate the verity of this underlying mechanism/interaction as the biological control of aphids was significantly enhanced (a 5 times reduction in aphid population) in the combination *Orius majusculus* – *A. aphidimyza* – thrips.

In contrast with the anthocorid bugs, IGP did not play a significant role in the combination with the mirid bug *Macrolophus caliginosus* – *A. aphidimyza*. Although gallmidge densities were slightly (but not significantly) lower in the presence of the mirid bug and predation was observed, the occurrence of IGP was rare. Additionally, no effects were found on aphid densities in this treatment.

However, when whiteflies were present, significant higher aphid densities were observed (a 12 times increase). As direct or indirect (plant-mediated or predator-mediated) interactions between aphids and whitefly felt beyond this study, the effect of whitefly is not clear. Direct interactions between aphids and whiteflies are not very plausible, as space was not a limiting factor. A possible explanation for the higher aphid densities in presence of whiteflies might be a plant-mediated indirect interaction between the two pest species. This was observed earlier in tomato where whitefly *Bemisia tabaci* induced plant responses (by changing plant quality), improving development of aphids (Nombela et al., 2008). Possible plant-mediated interactions between *Trialeurodes vaporariorum* and *Myzus persicae* need further research.

Finally, one must not forget the possibility of trait-mediated interactions by the alternative prey on *Macrolophus caliginosus* (changing foraging behaviour of the predatory bug; not its density) (Prasad & Snyder, 2006). As the aphid density increased in the presence of alternative prey, the enhanced foraging behaviour of *Macrolophus caliginosus* might have led to anti-predator behaviour by gallmidges, avoiding patches with shared prey and the intraguild predator (reducing aphid control). The possible occurrence of this trait-mediated indirect interaction needs further research too.

5 Conclusions

The results of this study demonstrate the presence of intraguild predation although it seems to have a low impact on aphid control by gallmidges on the short term. It is not known if this will be different on the long term, because of a lower population development of the gallmidges due to this IGP. The short-term effects are however much more representative for greenhouse biocontrol (i.e. damage must stay below the economical threshold).

The presence of alternative food for the predatory bugs is not found to affect IGP interactions although it is found to influence aphid development. In the combination ‘*Aphidoletes* – *Orius majusculus* – thrips’ aphid control was enhanced; thought to be due to TMII-interactions (the alternative prey enhances foraging behaviour of the predatory bug, increasing encounter rates with and predation of aphids). On the contrary, aphid control was negatively affected in the combination ‘*Aphidoletes* – *Macrolophus* – whitefly’. Plant-mediated indirect interactions between whitefly and aphids may be responsible for this observation. Also plausible is an enhanced foraging behavior of *Macrolophus*, causing anti-predator behaviour of gallmidges. The correctness of these opposed underlying mechanisms needs further research.

Gallmidges as additional food source, seem to enhance *Orius* development which may be beneficial for thrips control. On the other hand it may result in an increased IGP on the long term, negatively affecting aphid control and caution must be taken.

As IGP by predatory bugs does not seem to affect *Aphidoletes aphidimyza*, its bad establishment may be caused by other predators (e.g. predation by mites) or have to do with environmental factors, leaving clearance for further research.

Recommendations

Effect on aphid development

As IGP was not found to influence aphid control, the observed changes in aphid population in this study must be caused by other factors. The direct or indirect (plant-mediated or predator-mediated) interactions between aphids and thrips/whitefly need further research, such as:

1. Mutual interaction between whitefly and aphids might have caused the enhanced aphid development. This is supported by the study of Nombela et al. (2008), who found an enhanced aphid development on whitefly-infested plants. As this study worked with other whitefly/aphid species, it is of interest to know if this also holds for the species used in this study (i.e. *Trialeurodes vaporariorum* and *Myzus persicae*).
2. As the density of the predators was not affected, TMII between the predator and the alternative prey might have occurred, affecting aphid density indirectly (via increased foraging behaviour of the predatory bug/anti-predator behaviour of the gallmidges). Empirical evidence is needed to support these suggestions.

On the longer term...

In this study only short-term experiments were performed with one generation of *Aphidoletes aphidimyza*. One must note that in the production of greenhouse vegetables *Aphidoletes* is inserted periodically as successful pupation is difficult due to the lack of soil. However, as the predatory bugs survive and seem to grow in population size due to the presence of gallmidges, it could be that the second release of *Aphidoletes* suffers more and aphid control is influenced differently.

Gallmidges, a delicacy for other predators?

As more Orius larvae developed in the presence of gallmidges, it is of interest to know if gallmidges indeed enhance larvae development and how (i.e. additive nutritional value). A higher number of Orius could namely be beneficial for thrips control.

*Further research on *Aphidoletes aphidimyza**

From this study it became clear that the presence of predatory bugs does not cause a bad functioning of *Aphidoletes aphidimyza*. Environmental factors or other predators like predatory mites (Messelink, unpubl. data) might have a larger effect, but this must be further investigated.

Acknowledgements

I got the opportunity to do my thesis at *WUR Glastuinbouw* in Bleiswijk, which gave me not only the chance to get more experience with working within a practice-related company, but also kept me up to date of new and interesting developments in horticulture.

I would like to thank my major supervisor Ir. Gerben Messelink for his guidance and expertise during my thesis. I could always knock at his door if I had a question and he gave advice when I was struggling with odd results, keeping me on the right track. I would also like to thank Prof. Dr. Marcel Dicke for his comments and fast reaction on questions I had.

Finally, I am grateful to all the people of Plant health at *WUR Glastuinbouw* for their practical view and tips. The working atmosphere was really nice or ‘gezellig’, as we say in Dutch.



Figure 6.1: *Orius laevigatus* (adult)

References

- Albajes R. & Alomar Ò. (2000). Current and Potential use of Polyphagous Predators. *Integrated Pest and Disease Management in Greenhouse Crops*, 265-275.
- Alhmedi A., Francis F., Bodson B. & Haubruge E. (2007). Intraguild interactions of aphidophagous predators in fields: effect of *Coccinella septempunctata* and *Episyrphus balteatus* occurrence on aphid infested plants. *Communications in agricultural and applied biological sciences*. **72**(3): 381-390.
- Bilu E. & Coll M. (2007). The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. *BioControl*. **52**(6): 753-763.
- Bilu E., Hopper K. R. & Coll M. (2006). Host choice by *Aphidius colemani*: Effects of plants, plant-aphid combinations and the presence of intra-guild predators. *Ecological Entomology*. **31**(4): 331-336.
- Bonato O., Couton L. & Fargues J. (2006). Feeding Preference of *Macrolophus caliginosus* (Heteroptera: Miridae) on *Bemisia tabaci* and *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae). *Journal of Economic Entomology*. **99**(4): 1143–1151
- Brødsgaard H. F. & Enkegaard A. (1995). Interactions among polyphagous anthocorid bugs used for thrips control and other beneficials in multi-species biological pest management systems. *Med. Fac. Landbouw. Univ. Gent*. **60**(3a): 893-900.
- Capinera J. L. (2001). Green Peach Aphid, *Myzus Persicae* (Sulzer)(Insecta: Hemiptera: Aphididae). *Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida*. <http://creatures.ifas.ufl.edu>.
- Chacón J. M., Landis D. A. & Heimpel G. E. (2008). Potential for biotic interference of a classical biological control agent of the soybean aphid. *Biological Control*. **46**(2): 216-225.
- Choi M., Roitberg B. D., Shani A., Raworth D. A. & Lee G. H. (2004). Olfactory response by the aphidophagous gall midge, *Aphidoletes aphidimyza* to honeydew from green peach aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata*. **111**(1): 37-45.
- Christensen R. K., Enkegaard A. & Brødsgaard H. F. (2002). Intraspecific interactions among the predators *Orius majusculus* and *Aphidoletes aphidimyza*. *IOBC/wprs Bulletin*. **25**(1): 57-60.
- Daugherty M. P., Harmon J. P. & Briggs C. J. (2007). Trophic supplements to intraguild predation. *Oikos*. **116**: 662-677.

- El-Titik A. (1973). Einflüsse von Beutedichte und Morphologie der Wirtsplanze auf die Eialblage von *Aphidoletes aphidimyza* (Rond). (Diptera: Itonididae). *Zeitschrift für Angewandte Entomologie*. **72**: 400-415.
- Emden H. F. V., Eastop V. F., Hughes R. D. & Way M. J. (1969). The Ecology of *Myzus Persicae*. *Annual Review of Entomology*. **14**(1): 197-270.
- Enkegaard A. & Brødsgaard H. F. (2006). Biocontrol in protected crops: is lack of biodiversity a limiting factor? *In: An Ecological and Societal Approach to Biological Control*, 91-112.
- Enkegaard A., Christensen R. K. & Brødsgaard H. F. (2005). Interspecific interactions among the aphid parasitoid *Aphidius colemani* and the aphidophagous gallmidge *Aphidoletes aphidimyza*. *IOBC/WPRS Bulletin*. **28**(1): 83-86.
- Fréchette B., Rojo S., Alomar O. & Lucas E. (2007). Intraguild predation between syrphids and mirids: Who is the prey? Who is the predator? *BioControl*. **52**(2): 175-191
- Gardiner M. M. & Landis D. A. (2007). Impact of intraguild predation by adult *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Hemiptera: Aphididae) biological control in cage studies. *Biological Control* **40**(3): 386-395.
- Gillespie D. R. (*In press*). Importance of theory in the biological control of aphids in greenhouses. ISBCA conference 2009. Christchurch, New Zealand.
- Hemptinne J. L., Dixon A. F. G. & Gauthier C. (2000). Nutritive cost of intraguild predation on eggs of *Coccinella septempunctata* and *Adalia bipunctata* (Coleoptera: Coccinellidae). *European Journal of Entomology*. **97**(4): 559-562.
- Hindayana D., Meyhöfer R., Scholz D. & Poehling H. M. (2001). Intraguild Predation among the Hoverfly *Episyrphus balteatus* de Geer (Diptera: Syrphidae) and Other Aphidophagous Predators. *Biological Control*. **20**(3): 236-246.
- Hironori Y. & Katsuhiko S. (1997). Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. *Entomophaga*. **42**: 153-163.
- Holt R. D. & Polis G. A. (1997). A theoretical framework for intraguild predation. *The American Naturalist*. **149**(4): 745-764.
- Ito K. (2007). A simple mass-rearing method for predaceous Orius bugs in the laboratory. *Applied Entomology and Zoology*. **42**(4): 573-577.
- Janssen A., Montserrat M., HilleRisLambers R., Roos A., Pallini A. & Sabelis M. (2006). Intraguild Predation Usually does not Disrupt Biological Control. *In: Trophic and Guild in Biological Interactions Control*, 21-44.
- Janssen A., Sabelis M. W., Magalhaes S., Montserrat M. & Van der Hammen T. (2007). Habitat structure affects intraguild predation. *Ecology*. **88**(11): 2713-2719.

- Jazzar C., Meyhöfer R., Ebssa L. & Poehling H. M. (2008). Two protagonists on aphidophagous patches: Effects of learning and intraguild predation. *Entomologia Experimentalis et Applicata*. **127**(2): 88-99.
- Lucas E. (1998^a). How do ladybirds (*Coleomegilla maculata lengi*, Coleoptera: Coccinellidae) feed on green lacewing eggs (*Chrysoperla rufilabris*, Neuroptera: Chrysopidae). *Canadian Entomology*. **130**(547-548).
- Lucas E. & Brodeur J. (2001). A fox in sheep's clothing: furtive predators benefit from the communal defense of their prey. *Ecology*. **82**(11): 3246.
- Lucas E., Coderre D. & Brodeur J. (1998^b). Intraguild predation among aphid predators: Characterization and influence of extraguild prey density. *Ecology*. **79**(3): 1084.
- Malais M. H. & Ravensberg W. J. (2002). Kennen en herkennen: levenswijzen van kasplagen en hun natuurlijke vijanden. *Reed Business Information, Koppert*. ISBN: 9789054391197.
- Markkula M., Tiittanen K., Hamalainen M. & Forsberg A. (1979). The aphid midge *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae) and its use in biological control of aphids. *Annual Entomol Fenn*. **45**: 89-98.
- Meiracker R. A. F. (1999). Do functional responses of predatory arthropods reach a plateau? A case study of *Orius insidiosus* with western flower thrips as prey. *Entomologia experimentalis et applicata*. **90**(3): 323.
- Messelink G. J., van Maanen R., van Steenpaal S. E. F. & Janssen A. (2008). Biological control of thrips and whiteflies by a shared predator: Two pests are better than one. *Biological control*. **44**(3): 372.
- Meyling N. V., Enkegaard A. & Brødsgaard H. (2004). Intraguild predation by *Anthocoris nemorum* (Heteroptera: Anthocoridae) on the aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconidae) *Biocontrol Science and Technology*. **14**(6): 627-630.
- Montserrat M. (2000). Functional response of four heteropteran predators preying on greenhouse whitefly (Homoptera: Aleyrodidae) and western flower thrips (Thysanoptera: Thripidae). *Environmental Entomology*. **29**(5): 1075.
- Nakashima Y., Birkett M. A., Pye B. J., Pickett J. A. & Powell W. (2004). The role of semiochemicals in the avoidance of the seven-spot ladybird, *Coccinella septempunctata*, by the aphid parasitoid, *Aphidius ervi*. *Journal of Chemical Ecology*. **30**(6): 1103-1116.
- Nakashima Y., Birkett M. A., Pye B. J. & Powell W. (2006). Chemically mediated intraguild predator avoidance by aphid parasitoids: Interspecific variability in sensitivity to semiochemical trails of ladybird predators *Journal of Chemical Ecology*. **32**(9): 1989-1998.

- Nakashima Y. & Senoo N. (2003). Avoidance of ladybird trails by an aphid parasitoid *Aphidius ervi*: Active period and effects of prior oviposition experience *Entomologia Experimentalis et Applicata* **109**(2): 163-166.
- Nombela G., Garzo E., Duque M. & Muñiz M. (2008). Preinfestations of tomato plants by whiteflies (*Bemisia tabaci*) or aphids (*Macrosiphum euphorbiae*) induce variable resistance or susceptibility responses. *Bulletin of Entomological Research*.
- Phoofolo M. W. & Obrycki J. J. (1998). Potential for intraguild predation and competition among predatory Coccinellidae and Chrysopidae. *Entomologia Experimentalis et Applicata*. **89**: 47-55.
- Pineda A., Morales I., Marcos-Garcia M. A. & Fereres A. (2007). Oviposition avoidance of parasitized aphid colonies by the syrphid predator *Episyrphus balteatus* mediated by different cues. *Biological Control*. **42**(3): 274-280.
- Polis G. A. & Holt R. D. (1992). Intraguild predation: The dynamics of complex trophic interactions. *Trends in ecology & evolution*. **7**(5): 151.
- Polis G. A., Myers C. A. & Holt R. D. (1989). The Ecology and Evolution of Intraguild Predation: Potential Competitors That Eat Each Other. *Annual review of ecology and systematics*. **20**(1): 297.
- Prasad R. P. & Snyder W. E. (2006). Diverse trait-mediated interactions in a multi-predator multi-prey community. *Ecology*. **87**(5): 1131-1137.
- Rabasse J. & Steenis M. (2000). Biological Control of Aphids. *In: Integrated Pest and Disease Management in Greenhouse Crops*, 235-243.
- Rosenheim J. A. (1995). Intraguild Predation Among Biological-Control Agents: Theory and Evidence. *Biological control*. **5**(3): 303.
- Rosenheim J. A., Wilhoit L. R. & Armer C. A. (1993). Influence of Intraguild Predation among Generalist Insect Predators on the Suppression of an Herbivore Population. *Oecologia*. **96**(3): 439-449.
- Santi F. & Maini S. (2006). Predation upon *Adalia bipunctata* and *Harmonia axyridis* eggs by *Chrysoperla carnea* larvae and *Orius leavigatus* adults. *Bulletin of insectology*. **59**(1): 53-58.
- van Lenteren J.C., Roskam M.M. & Timmer R. (1997). Commercial Mass Production and Pricing of Organisms of Pests in Europe. *Biological control*. **10**: 143-149.
- van Lenteren J. C. (2000). A greenhouse without pesticides: fact or fantasy? *Crop protection*. **19**(6): 375.
- van Schelt J. (2000). Improved methods of testing and release of *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) for aphid control in glasshouses. *European journal of entomology*. **97**(4): 511.

- Venzon M., Janssen A. & Sabelis M. W. (2001). Prey Preference, Intraguild Predation and Population Dynamics of an Arthropod Food Web on Plants. *Experimental and Applied Acarology*. **25**(10/11): 785-808.
- Yano E. (2006). Ecological considerations for biological control of aphids in protected culture. *Population ecology*. **48**(4): 333.