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Citation for published version (APA):
Messelink, G. J. (2012). Generalist predators, food web complexities and biological pest control in greenhouse crops

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G.J. Messelink, 2012. Generalist predators, food web complexities and biological pest control in greenhouse crops

PhD thesis, University of Amsterdam, The Netherlands

Publication of this thesis was financially supported by Koppert Biological Systems (www.koppert.nl)

ISBN: 978 94 91407 04 8

Lay-out: Jan Bruin (www.bred.nl)

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

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D.C. van den Boom
ten overstaan van een door het college voor promoties
ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel

op dinsdag 5 juni 2012, te 14:00 uur

door

Gerben Jaap Messelink

geboren te Doornspijk

Promotiecommissie

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Introduction

Biological control of pest species has traditionally mainly focused on specific natural enemies for each pest (Huffaker & Messenger, 1976; Hokkanen & Pimentel, 1984; van Lenteren & Woets, 1988; Hoy, 1994). However, pest-enemy interactions are often embedded in rich communities of multiple interacting pests and natural enemies (e.g., Helle & Sabelis, 1985b; Minks & Harrewijn, 1989; Sabelis, 1992), and the interactions among these species affect the efficacy of biological control (Sih et al., 1985; Janssen et al., 1998; Prasad & Snyder, 2006; Evans, 2008). The effect of interactions among various species of predators and parasitoids on biological control of a shared pest species has received ample attention (see Letourneau et al., 2009), showing that it can range from larger to smaller than the effect of each enemy species separately (Rosenheim et al., 1995, 1998; Losey & Denno, 1998; Colfer & Rosenheim, 2001; Venzon et al., 2001; Cardinale et al., 2003; Snyder & Ives, 2001, 2003; Finke & Denno, 2004; Cakmak et al., 2009). However, it is not only predator diversity, but also the diversity of herbivorous prey that may affect the suppression of a particular pest species through competition, or indirect interactions mediated by host plant or shared predators (Holt, 1977; Karban & Carey, 1984). Hence, designing effective biological control programs for more than one pest species requires an understanding of all interactions occurring among species within biocontrol communities, not just those among pests and their natural enemies or among different species of natural enemies.

Greenhouse crops are often considered as simple ecosystems with low biodiversity (Enkegaard & Brødsgaard, 2006). Especially modern greenhouses appear sterile compared to outdoor crops, as plants are grown on hydroponic systems in greenhouses that are isolated from the environment because of modern energy saving techniques (Bakker, 2008). However, the general experience is that infestations by several small pest species cannot be avoided, and the release of natural enemies against these pests adds to the diversity (van Lenteren et al., 2000; Cock et al., 2010). Thus, apparently ‘clean’ greenhouse crops often accommodate complex artificial communities of multiple pests and natural enemies. Furthermore, there seems to be a tendency that the diversity of these communities increased during the last decades (Enkegaard & Brødsgaard, 2006). One reason for this increased diversity is the invasion of exotic pest species (global trade, global warming) (Roques et al., 2009). Second, more species than before develop into pests as a result of the reduced use of pesticides and the use of more selective pesticides (van der Blom et al., 2009; Pijnakker & Leman, 2011). A third reason is that biological control programs increasingly include generalist predators (Gerson & Weintraub, 2007; Sabelis et al., 2008), and such generalists potentially interfere more with other natural enemies than specialists. Thus, recent developments further increase food web complexity in biological control programs and emphasize that such complexities need to be considered when designing biological control programs. The advantage of greenhouse crops is that they offer the unique possibility to create the desired communities of natural enemies by choosing and releasing natural enemies out of the many species that are commercially available nowadays (van Lenteren, 2000; Enkegaard & Brødsgaard, 2006). In other words, biodiversity can be created and manipulated to maximise sustainable pest control. At the same time, such systems can be used to study the manipulation of biodiversity on the dynamics of communities of plant-inhabiting arthropods under relatively controlled conditions and at larger spatial scales than can usually be realized with communities under field conditions.

Here, I review the ecological theory relevant to interactions in food webs occurring within arthropod communities and I discuss the possible implications for biological control in greenhouses. The subsequent chapters contain studies that focus on the most important greenhouse pests, namely aphids, thrips, spider mites and whiteflies, as well as their natural enemies (see BOXES with pest descriptions).

Food web theory and effects in greenhouse crops

Consumption (i.e., herbivory, predation and parasitism) and competition are considered the two most important interactions determining the structure of communities (Chase et al., 2002). Within communities of natural enemies and pests, species may interact through exploitative competition, through predation and parasitism (including omnivory, intraguild predation and hyperpredation or hyperparasitism), but also through apparent competition or apparent mutualism via shared natural enemies (FIGURE 1.1). Besides these density-mediated interactions, species interactions can be modified through trait changes of the interacting individuals (which includes changes in behaviour and induced plant responses). In the following, I summarize the current theory on these interactions and their relevance to biological control.

Exploitative competition and induced plant responses

Herbivores can interact through exploitative competition for the plant (FIGURE 1.1), but this is undesirable for biological control because it occurs at high pest densities, which may exceed the economic damage threshold. I will therefore refrain from discussing resource competition among herbivores here.

Herbivores can also interact via the plant when the attack of one species induces defence responses in the plant that also affect a second species (Karban & Carey, 1984). These plant responses can result in either increased resistance or increased susceptibility (e.g., Karban & Baldwin, 1997; Sarmento et al., 2011a). Plant resistance against insects consists of direct defences, such as the production of toxins and feeding deterrents that reduce survival, fecundity or reduce developmental rate (Kessler & Baldwin, 2002), and indirect defences such as the production of plant volatiles that attract carnivorous enemies of the herbivores (Dicke & Sabelis, 1988; Schaller, 2008). Several biochemical pathways are involved in these processes (Walling, 2000). Recent studies have shown that plant-mediated interactions between herbivores are very common and could be important in structuring herbivore communities (Kessler et al., 2007). Models of interactions that are mediated by inducible changes in plant quality predict a range of outcomes including coexistence, multiple equilibria, dependence on initial conditions and competitive exclusion of some herbivore species (Anderson et al., 2009). It should be noted that these models assume that herbivore populations are well mixed and possible variation in induction of plant defences caused by variation in population densities is ignored.

Several studies documented indirect interactions between herbivores through induced changes in plant quality (Karban & Baldwin, 1997), but studies on green-

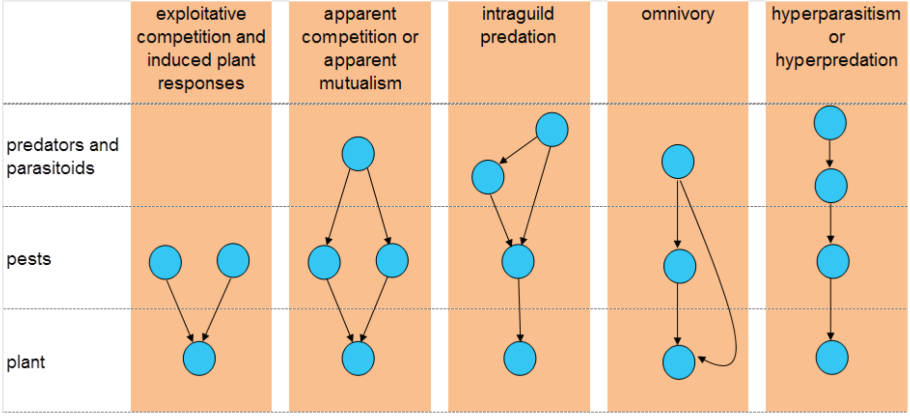

FIGURE 1.1 – Schematic diagrams of the direct and indirect interactions among plants, pests and natural enemies that will be treated in this chapter. Arrows indicate consumption. From left to right: *exploitative competition* in which two pest species compete for the same plant, but also affect each other's densities through induced plant defences. *Apparent competition or apparent mutualism* refers to indirect interactions between two prey species mediated by a shared natural enemy (with pests on the same plants this automatically includes exploitative competition and induced plant defences). *Intraguild predation* refers to predators consuming another natural enemy with whom they also compete for the same pest species. *Omnivory* means consumption on more than one trophic level ('true' omnivores are predators that feed on both pests and plants). *Hyperparasitism or hyperpredation* represents the consumption of natural enemies by other natural enemies with whom they do not compete for shared prey, but they differ by the fact that hyperpredators can develop on alternative prey, whereas true hyperparasitoids are obligate. Except for induced plant responses, these interactions are density mediated.

house crops are limited. In tomato, it has been demonstrated that infestations by caterpillars of a noctuid moth increased resistance to spider mites, aphids and another lepidopteran pest (Stout et al., 1998). Likewise, infestation of tomato and cucumber plants by whiteflies induced resistance against leaf miners (Inbar et al., 1999; Zhang et al., 2005). Induced susceptibility may also occur, for example, infestations of tomato plants by whiteflies increased susceptibility to aphids (Nombela et al., 2009). On lima bean, similar results were found for whiteflies and spider mites (Zhang et al., 2009). The spider mite *Tetranychus evansi* Baker & Pritchard was found to down-regulate plant defences (Sarmento et al., 2011a), and the closely related species *Tetranychus urticae* Koch can profit from this induced susceptibility (Sarmento et al., 2011b). Induced resistance may also affect the behaviour of omnivores that facultatively feed on plants. The omnivorous Western flower thrips switched from feeding on cotton plants to feeding on spider mite eggs when defences of the plants were induced (Agrawal et al., 1999). Moreover, they performed worse on a diet of spider mite eggs from induced plants as opposed to non-induced plants (Agrawal & Klein, 2000). In conclusion, plant-mediated interactions among pest species are probably a common phenomenon in greenhouse crops, where they may influence the biological control of multiple pests.

Apparent competition and apparent mutualism

Generalist predators can mediate indirect interactions among prey species that might otherwise not interact (Holt & Lawton, 1994; Janssen et al., 1998; Harmon & Andow, 2004; van Veen et al., 2006) (FIGURE 1.1). If, for example, the carrying capacity of one prey species increases, this results in an increased equilibrium density of the shared predator and a decreased equilibrium density of the second prey species. Holt (1977) suggested the term 'apparent competition' for this interaction between prey species, because the dynamics of the two species resemble that of species competing for resources, whereas in fact it is mediated by the shared predator (see BOX Apparent competition). Apparent competition is usually defined as a reciprocal negative interaction between prey species. Most empirical studies, however, show non-reciprocal indirect interactions; only one of the two prey species is negatively affected by the predator-mediated prey interaction (Chaneton & Bonsall, 2000). Originally, the theory of apparent competition considered equilibrium densities. However, generalist predators can also cause 'short-term' apparent competition between prey species when predators aggregate in habitat patches containing both prey, or when their feeding rate on one prey is enhanced by the presence of another prey (Holt & Kotler 1987; Müller & Godfray 1997).

The opposite effect may also occur: two prey species that share a natural enemy may also affect each other's density positively (apparent mutualism). This occurs

BOX | Apparent competition

Apparent competition considers the indirect interaction between two prey species (N_1 and N_2) that share a predator (P) (Holt, 1977). As an example, I consider two prey species, each with logistic population growth, and a predator population that shows a linear functional response to the densities of the two prey species:

$$\frac{dN_1}{dt} = N_1 r_1 \left(1 - \frac{N_1}{K_1}\right) - a_1 N_1 P$$
$$\frac{dN_2}{dt} = N_2 r_2 \left(1 - \frac{N_2}{K_2}\right) - a_2 N_2 P$$
$$\frac{dP}{dt} = b_1 N_1 P + b_2 N_2 P - mP.$$

The per capita growth rate is represented by r_i and the carrying capacity of the prey by K_i. The per capita predation rate is represented by a_i and the per capita population growth rate of the predators by b_i, which can be thought of as a product of the per capita predation rate a_i and the per capita rate of conversion of consumed prey to predators. The per capita mortality rate of the predators is represented by a constant, m. The equilibrium densities can be calculated by setting $dN_i/dt = 0$ and $dP/dt = 0$. These equilibria have been shown to be stable (Holt, 1977).

For the special case where the predation rate on each of the two prey is equal ($a_1 = a_2$) and growth rates of prey and predator are equal ($r_1 = r_2$; $b_1 = b_2$), the equilibrium densities are:

$$N_1^* = \frac{mK_1}{b(K_1 + K_2)}$$
$$N_2^* = \frac{mK_2}{b(K_1 + K_2)}$$
$$P^* = \frac{r}{a}\left[1 - \frac{m}{b(K_1 + K_2)}\right].$$

This shows that the quilibrium densities of the prey depend on each other's carrying capacity: an increase in the carrying capacity of one prey species (or the addition of a second species to a system of only 1 prey and 1 predator) will decrease the equilibrium density of the other prey species. Such a change is expected when the two prey species compete for the same resource, but this is not the case in the model and therefore their competitive relation must be apparent, i.e., it looks like competition, but results from another mechanism. In fact, as can be seen from the expression for P^*, an increase in the carrying capacity of one prey species causes an increase the predator equilibrium densities and thereby decreases the equilibrium density of the other prey species. This can be easily seen from the differential equation of P at equilibrium ($dP/dt = 0$), which yields $P^* = 0$ or: $b_1 N_1^* + b_2 N_2^* = m$. From this it can be seen directly that an increase in the equilibrium density of one prey species results in a decrease of the density of the other species.

when increases in the density of one prey species result in satiation of the shared predator. Increases in the density of one prey species can also result in the predator changing from feeding predominantly on a second prey to feeding mainly on the first prey (switching, Murdoch, 1969), consequently reducing the consumption of the second prey species (Abrams & Matsuda, 1996). This effect is apparent in the short-term, when the densities have not yet reached an equilibrium (transient dynamics), because eventually, the predator populations will increase because of the higher densities of prey, resulting in apparent competition (Abrams & Matsuda, 1996). Apparent mutualism may also occur in the long term when population densities do not reach equilibria, but show cycles, causing repeated satiation of the shared predators and repeated reduced predation on the other prey (Abrams et al., 1998). Hence, depending on the time scale and on the type of dynamics, theory predicts that a shared natural enemy can generate positive or negative indirect effects between prey species.

Apparent competition and apparent mutualism are inherently related to diet choice and switching of the predators from feeding on one prey to feeding on the other prey or on both prey, but effects of mixed diets on predator performance are also relevant. Mixed diets are known to have positive effects on reproduction in some predator species (Wallin et al., 1992; Evans et al., 1999; Toft & Wise, 1999). So far, this aspect of mixed diets has been ignored in theoretical models about apparent competition. Basic models about apparent competition assume that each prey species is suitable for reproduction of the shared natural enemy (Holt, 1977). However, it is also possible that two prey species vary greatly in suitability for the shared natural enemy, for example parasitoids may only marginally develop in some hosts. A model for parasitoids showed that in such cases, the suitable host can benefit from the presence of the marginal host (assuming no evolution of host preference), but the marginal host suffers from the presence of the suitable host (Heimpel et al., 2003). Observations on aphid parasitoids confirm that unsuitable hosts are indeed attacked by parasitoids in the presence of suitable hosts, which was detrimental for the foraging efficiency of the parasitoid (Meisner et al., 2007).

When generalist predators are released in greenhouse crops, pest species such as thrips, whiteflies, spider mites and aphids can be involved in apparent competition or apparent mutualism. Examples of such generalist predators are anthocorid and mirid bugs and several species of predatory mites. Only few studies on these indirect predator-mediated interactions exist, and they all focused on short-term effects, showing that presence of one pest can release another pest from control (Xu et al., 2006; Desneux & O'Neil, 2008). Although the theory of predator-mediated interactions has long been ignored in many biological control studies, there has been a long-standing interest in the use of alternative prey species for enhancing biological control (Stacy, 1977). The method by which these alternative prey species or food are facilitated is based on the introduction of a non-crop plant harbouring the alternative prey species or providing alternative food sources. It is often referred to as the ‘banker plant method’ (Frank, 2010; Huang et al., 2011). A widely applied system in greenhouse crops is the use of monocotyledonous plants with grain aphids that serve as alternative hosts for parasitoids of aphids that attack the crop (Huang et al., 2011). The elegance of this system is that the grain aphids are host specific and pose no threat to the crop. Plants that produce a lot of pollen may serve as banker plants for generalist predators (Ramakers & Voet, 1995). For example, pollen can serve as food for generalist predatory mites and enhance the biological control of thrips and whiteflies on cucumber (van Rijn et al., 2002; Nomikou et al., 2010). In fact, all kinds of ‘open rearing’ systems of natural enemies in greenhouse crops (e.g., rearing sachets containing small cultures of predatory mites, bran and astigmatic mites) are based on the principles of apparent competition, but there is little awareness that apparent mutualism may also occur.

Intraguild predation

Natural enemies can compete for the same prey species, but this is frequently combined with predation by one species of natural enemy on another (Rosenheim et al., 1995), which is called intraguild predation (IGP, FIGURE 1.1). The predator that kills and eats the other natural enemy is called the intraguild predator and the other natural enemy is the intraguild prey (Polis et al., 1989; Holt & Polis, 1997).Theory predicts that IGP can only result in stable coexistence of the species when the intraguild prey is the superior competitor for the shared prey, and only in systems with intermediate levels of productivity (Holt & Polis, 1997). These conditions are very restrictive and thus predict that IGP is not common in nature. However, it has become clear that IGP generally occurs in many ecosystems, including biological control systems (Polis et al., 1989; Rosenheim et al., 1995; Janssen et al., 2006, 2007). There may be several reasons for this discrepancy between theory, predicting that systems with strong IGP will be rare, and reality, where IGP is common. Factors that can contribute to the coexistence of intraguild predators and intraguild prey are now increasingly included in theoretical models. Examples of such factors are structured populations with intraguild prey stages that are invulnerable or intraguild predator stages that do not prey on the other predator (Mylius et al., 2001), anti-predator behaviour (Heithaus, 2001), switching intraguild predators (Krivan, 2000) or alternative prey (Daugherty et al., 2007; Holt & Huxel, 2007).

Intraguild predation has been described for many natural enemies that are used for biological control in greenhouse crops (Rosenheim et al. 1995; Janssen et al., 2006). Based on theory, intraguild predation is expected not to benefit biological control (Rosenheim et al., 1995), but in practice, results are mixed (Janssen et al., 2006, 2007; Vance-Chalcraft et al., 2007). Here, I summarize the occurrence of intraguild predation among natural enemies of thrips, whiteflies, aphids and spider mites. The omnivorous predator *Macrolophus pygmaeus* (Rambur) (formely identified as *Macrolophus caliginosus* Wagner) is an intraguild predator of natural enemies of aphids; it consumes the eggs of the syrphid *Episyrphus balteatus* de Geer (Frechette et al., 2007) and parasitized aphids (Martinou et al., 2009). This predator did not prey on nymphal stages of *Orius majusculus* (Reuter), but in turn, the nymphal stages of *M. pygmaeus* were vulnerable for predation by *O. majusculus* (Jakobsen et al., 2004). Predatory bugs from the genus *Orius* act as intraguild predators of phytoseiid mites (Gillespie & Quiring, 1992; Venzon et al., 2001; Brødsgaard & Enkegaard, 2005; Chow et al., 2008), the aphidophagous predatory midge *Aphidoletes aphidimyza* (Rondani) (Hosseini et al., 2010) and aphid parasitoids (Snyder & Ives, 2003). Many generalist predatory mites are intraguild predators of other predatory mites (Schausberger & Walzer, 2001; Montserrat et al., 2008; Buitenhuis et al., 2010; van der Hammen et al., 2010) or juvenile stages of predatory bugs (Madali et al., 2008). Finally, a number of studies show intraguild predation among specialist natural enemies of aphids. The syrphid *E. balteatus* feeds on freshly parasitized as well as unparasitized aphids (Brodeur & Rosenheim, 2000). Syrphid larvae may also consume the aphidophagous gall midge *A. aphidimyza*, but predation rates are low in the presence of aphids (Hindayana et al., 2001). In turn, this midge does not prey on *E. balteatus* (Hindayana et al., 2001), but may consume parasitized aphids (Brodeur & Rosenheim, 2000).

None of the studies mentioned above demonstrate a negative effect of intraguild predation on biological control in greenhouse crops. Although the potential risk of intraguild predation disrupting biological control appears to be low in many cases (Janssen et al., 2006), there are also examples of negative effects of intraguild predation on biological control.

Omnivory

Omnivory in its broadest sense can be defined as the consumption of species at more than one trophic level. Under this definition, intraguild predators are also omnivores. Predators that feed on both animals and plants are a particular case of trophic omnivory, also referred to as ‘true omnivory’ (Coll & Guershon, 2002). The first theoretical models on its dynamical consequences showed that omnivory destabilizes food webs (Pimm & Lawton, 1978), which is remarkable, considering the fact that omnivory is a common interaction in food webs (Coll & Guershon, 2002; Polis & Strong, 1996). More specific theory for plant-feeding omnivores shows that omnivores can stabilize the dynamics and persistence of populations by switching between consuming plants and prey, especially when the searching efficiency of the

BOX | Thrips

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most important pest species in greenhouse crops in Europe and North America (Lewis, 1997; Shipp & Ramakers, 2004). Damage of plants results from both feeding on leaves, flowers and fruits, as well as by transmission of viruses. Although *F. occidentalis* is primarily considered a phytophagous species that feeds on plant tissue, plant nectar or pollen, it is actually an omnivore. The larvae and adults facultatively feed on spider mite eggs (Trichilo & Leigh, 1986), on predatory mite eggs (Faraji et al., 2001; Janssen et al., 2003), or on whitefly crawlers (van Maanen et al., in press). Biological control of Western flower thrips in greenhouses started with mass releases of the phytoseiid predatory mite *Neoseiulus barkeri* (Hughes) (= *Amblyseius mckenziei*) (Ramakers, 1980). Phytoseiid mites are still the most important thrips predators in many greenhouse crops nowadays (Gerson & Weintraub, 2007). Most of them are omnivorous; they do not only feed on thrips, but also on other prey, as well as and plant provided food such as pollen (for overviews see McMurtry & Croft, 1997; Gerson & Weintraub, 2007). A second important group of thrips predators are anthocorid bugs. The species most used in Europe is *Orius laevigatus* (Fieber), in Northern America it is *Orius insidiosus* (Reuter) (Brødsgaard, 2004; Shipp & Ramakers, 2004). These predators are released especially in (sweet) pepper crops where the continuous presence of pollinating flowers supplies sufficient food for establishment of predator populations even when prey is scarce (van den Meiracker & Ramakers, 1991). Although anthocorid bugs are mainly released for thrips control, they can also contribute to the control of whiteflies (Arnó et al., 2008), aphids (Alvarado et al., 1997; Butler & O'Neil, 2007), Lepidoptera species (Jacobson & Kring, 1994) and spider mites (Janssen et al., 1998; Venzon et al., 2002). Finally, thrips are sometimes controlled through releases of soil-dwelling predatory mites of the family of *Laelapidae* or *Macrochelidae*, which feed on the pupae of thrips in the soil (Gillespie & Quiring, 1990; Berndt et al., 2004; Messelink & van Holstein, 2008).

predator for prey is low relative to that for plant tissue (Lalonde et al., 1999). Hence, this theory suggests that biological control with plant-feeding omnivores may stabilize pest population dynamics. The question is, whether these equilibrium densities are acceptable for pest control (Lalonde et al., 1999). Other aspects of plant-feeding omnivory, such as the persistence of predators in the absence of prey, or the nutritional benefits for predators of feeding on plants may also result in positive contributions to biological control.

Many predators that are used for biological control are true omnivores, feeding on pests and plant-provided food such as pollen, nectar and plant saps. For example, many generalist predatory mites and bugs can complete their life cycle when feeding on pollen. However, not all greenhouse crops produce edible pollen, but some omnivores, such as the mirid bug *M. pygmaeus*, can also live and reproduce on plant saps. Although considered as a pest species, Western flower thrips, *Frankliniella occidentalis* (Pergande) are in fact omnivorous predators that feed on spider mites, predatory mites, whiteflies and plants (Trichilo & Leigh, 1986; Faraji et al., 2001; Janssen et al., 2003; van Maanen et al., in press). The consumption of prey in addition to plant material by mirid bugs and thrips can increase reproduction and developmental rates of these omnivores (Janssen et al., 2003; Perdikis & Lykouressis, 2004). The quality of the host plant can affect the predation rates of omnivores on pests (Agrawal et al., 1999; Agrawal & Klein, 2000; Magalhães et al., 2005; Hatherly et al., 2009) or the extent to which intraguild predation occurs (Janssen et al., 2003; Shakiya et al., 2009). Thus for biological control with predators that can also feed on the plant, it is important to known that the dynamics will be affected by plant quality.

Hyperpredation and hyperparasitism

In contrast to intraguild predation, natural enemies can also be consumed by other predators or parasitoids without sharing a prey with these enemies. Thus there is no competition for prey between the natural enemies. In parasitoids, the dynamical consequences of this so-called hyperparasitism are well-studied, both theoretically (Beddington & Hammond, 1977; May & Hassell, 1981) and empirically (Sullivan & Völkl, 1999). These studies indicate that obligate hyperparsitoids (secondary parasitoids that can develop only in or on a primary parasitoid) always lead to an increase of the pest equilibria, which might be detrimental to biological control. In case the hyperpredator is a true predator, there is no agreement in the literature on the name of this type of interaction. Some prefer to use the term 'secondary predation' (Rosenheim et al., 1995), or 'higher-order predation' (Rosenheim, 1998; Symondson, 2002) for predators consuming other predators, which includes both hyperpredation and intraguild predation. Even more confusing is that some interactions are described as hyperpredation, whereas it would be more consistent to typify them as apparent competition (e.g., Courchamp et al., 2000; Roemer et al., 2001) or intraguild predation (e.g., Roemer et al., 2002). In this thesis, I suggest to use the term hyperpredation in cases where predators eat other predators without sharing a prey, because of its similarity to hyperparasitism. However, an important difference is that hyperpredators can develop on alternative prey or food, whereas most hyperparasitoids specifically reproduce on or in other parasitoids. In the presence of alternative prey, hyperpredation can be classified as apparent competition between the alternative prey and the specialist natural enemy. To my knowledge, no specific the-

BOX | Whiteflies

Whiteflies are among the most important pest species of agricultural crops world wide. The species that cause damage to greenhouse crops are the polyphagous tobacco whitefly *Bemisia tabaci* Gennadius and the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood). In Northern European greenhouses, the dominant species is the greenhouse whitefly, which was the reason to use this species in this thesis. Whitefly damage is caused by nymphs and adults feeding on phloem, but also through the honeydew produced by the feeding stages, which contaminates the leaves. This facilitates the growth of sooty mold, which reduces photosynthesis (Byrne & Bellows, 1991). Biological control of *T. vaporariorum* in greenhouses started in the UK with the parasitoid *Encarsia formosa* Gahan (Speyer, 1927) and this wasp is still one of the biggest success stories in greenhouse biological control (van Lenteren, 2000). Since then, many other arthropods have been described as natural enemies of whiteflies, but only few species are applied commercially (Gerling et al., 2001). The most important species in greenhouse crops are the parasitoids *E. formosa*, *Eretmocerus mundus* Mercet and *Er. eremicus* Rose & Zolnerowich, the mirid bug *Macrolophus pygmaeus* (Rambur) (formely identified as *Macrolophus caliginosus* Wagner) and the predatory mite *Amblyseius swirskii* Athias-Henriot (Nomikou et al., 2002; Cock et al., 2010). The generalist predator *M. pygmaeus* is released mainly to control whiteflies (Gerling et al., 2001), although it has been observed to contribute to the control of aphids (Alvarado et al., 1997; Fantinou et al., 2009), thrips (Riudavets & Castañé, 1998; Blaeser et al., 2004), spider mites (Hansen et al., 1999), leaf miners (Arnó et al., 2003) and Lepidoptera species (Urbeja et al., 2009).

ory has been formulated on the effects of hyperpredation on prey populations in the presence of alternative prey. Theory on apparent competition predicts that the presence of one prey lowers the equilibrium densities of the second prey. For hyperpredation, this would mean that increases in the densities of the alternative prey will results in lower equilibrium densities of the specialist natural enemy, which would consequently release the prey of the specialist from control. In the short-term, satiation effects of the hyperpredator might result in apparent mutualism between the alternative prey and the specialist natural enemy. Hence, there will be a reduced negative effect on pest control by the specialist natural enemy.

In greenhouse crops, predatory mites that are used for control of thrips have been observed to be hyperpredators. They feed on eggs of predatory midges, but not on aphids, the pest that is controlled by larvae of predatory midges (van Schelt & Mulder, 2000). The impact of this type of interaction will receive more attention in this thesis. Hyperparasitism is common in the biological control of aphids in greenhouses and can disrupt biological control (Messelink, personal observations).

Effect of flexible behaviour

The interactions occurring in food webs that were described above all concern density-mediated interactions among species. However, it is generally recognized that traits of individuals, such as behaviour or defence, can change in response to the presence of individuals of other species (so-called trait-mediated interactions, Werner & Peacor, 2003). For example, anti-predator behaviour can strengthen or weaken density-mediated effects (Prasad & Snyder, 2006; Janssen et al., 2007). Many of these behavioural changes are mediated by chemical cues, which are released or left behind by both natural enemies and prey (Dicke & Grostal, 2001). Theoretical models of community dynamics now increasingly try to study the consequences of these behavioural-mediated interactions (e.g., Holt & Kotler, 1987; Abrams, 2008). These models show that the effects of such interactions may change the dynamics of the interacting species substantially.

Many interactions among natural enemies and pests in greenhouses can be affected by changes in the behaviour of pest and natural enemy. First of all, it is known that pest species can avoid their enemies. For example, whiteflies can learn to avoid plants with generalist predatory mites (Nomikou et al., 2003) and spider mites avoid plants with the predator *Phytoseiulus persimilis* Athias-Henriot (Pallini et al., 1999) or with thrips, which is a competitor and intraguild predator (Pallini et al., 1997). Aphids are well-known for their antipredator responses. For example, they kick at natural enemies, or they walk away or drop off the plants when perceiving a natural enemy (Villagra et al., 2002). Aphids as well as thrips release alarm pheromones that alert conspecifics (Bowers et al., 1972; Teerling et al., 1993; de Bruijn et al., 2006). Thrips can avoid predation by predatory bugs and predatory mites by using spider mite webbing as a refuge (Pallini et al., 1998; Venzon et al., 2000). They can defend themselves against predators by swinging with their abdomen and producing defensive droplets (Bakker & Sabelis, 1989), or even by counter-attacking the vulnerable egg stages of their phytoseiid predators (Faraji et al., 2001; Janssen et al., 2002). Natural enemies also respond to threats of other (intraguild) predators or counter-attacking prey. Predatory mites avoid ovipositing near counter-attacking thrips (Faraji et al., 2001) or intraguild predators (Choh et al., 2010; van der Hammen et al., 2010), or retain eggs in the presence of intraguild predators (Montserrat et al., 2007). Aphid parasitoids are known to avoid intraguild predation once they detect the chemical cues of the intraguild predators (Nakashima et al., 2006). The effects of intraguild predation can also be changed by the prey preference of the intraguild predator. For example, the syrphid *E. baltea-*

BOX | Spider mites

The most important spider mite pest in greenhouse crops is the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) (Gillespie & Raworth, 2004). This polyphagous pest causes damage to plants by puncturing the plant cells and feeding on their contents (Helle & Sabelis, 1985a). Moreover, they produce protective silk webs (Sabelis & Bakker, 1992), which eventually can completely cover infested plants. Biological control of spider mites with the specialist predatory mite *Phytoseiulus persimilis* Athias-Henriot is one of the cornerstones of biological control in greenhouse crops (Bravenboer & Dosse, 1962; Hussey & Bravenboer, 1971). Many other commercially applied phytoseiid predators feed on spider mites, but they are less specialized and feed on other prey as well (McMurtry & Croft, 1997). Other generalist predators, mentioned above, may also contribute to the suppression of spider mites in greenhouse cops, but most of them are hindered by the dense web produced by the spider mites (Sabelis & Bakker, 1992).

tus is an intraguild predator of aphid parasitoids because it consumes parasitized aphids, but when given a choice, it prefers to oviposit in aphid colonies without parasitized aphids (Pineda et al., 2007), thus weakening the effects of intraguild predation.

Interactions among species may change over time through learning or experience (Nomikou et al., 2003). For example, the predatory bug *O. majusculus* was more successful at preying on aphids after learning how to avoid the prey's kicking response (Henaut et al., 2000). Furthermore, predation rates on a specific pest might change through the presence of alternative food: the predatory bug *O. laevigatus* increased the predation rates on thrips in the presence of pollen (Hulshof & Linnamäki, 2002). Thus somehow, the pollen seemed to stimulate the feeding behaviour of these predators. In contrast, the presence of unsuitable prey may reduce the efficacy of a natural enemy for the target pest. For example, studies with parasitoids demonstrated that spending foraging time or eggs on less-suitable hosts will decrease parasitoid foraging success and ultimately decrease parasitoid population size (Meisner et al., 2007). Such effects may also occur in greenhouses when mixtures of aphid species are present in a crop. The reason why parasitoids attack unsuitable or marginal hosts in the study by Meisner et al. (2007) is not clear, perhaps the parasitoids and marginal hosts have not coevolved and there has been no selection on the parasitoid to discriminate between the marginal host and other host species. The examples presented above show that multiple prey effects can change the behaviour of shared natural enemies and may determine the outcomes of biological control.

Summarizing, changes in interactions or interaction strengths through flexible behaviour are common among the pests and natural enemies in greenhouse crops. Thus, when designing and interpreting results of multi-species experiments, it should be realized that both density-mediated interactions and behaviour-mediated interactions affect biological control.

Conclusions food web theory

Food web theory can provide insight into how various interactions between species might affect species dynamics and their possible effects on biological control. However, since models are based on simplifying assumptions, theoretical predictions are bound to differ from empirical studies (e.g., Janssen et al., 2006; Rosenheim & Harmon, 2006). One important reason for this is that theory is often tailored to predict equilibrium dynamics, whereas biological control systems often concern short-term (transient) dynamics, which might differ from long-term dynamics (Bolker et al., 2003; Briggs & Borer, 2005). A second reason is that food webs in reality are much more complex than theoretical models assume (Rosenheim et al., 1995; May, 1999; Coll & Guershon, 2002; Bolker et al., 2003; Cardinale et al., 2003; Janssen et al., 2006, 2007; Letourneau et al., 2009). The presence of multiple pests and natural enemies will result in joint effects of several types of interactions, and there is limited theory that takes such complexity into account. Furthermore, most models assume that populations are well mixed, whereas in reality arthropod populations are often clustered either within plants or within crops. To close these gaps between theory and practice, more long-term experiments are needed to observe dynamics of natural enemies and pest species over a sufficient number of generations to allow reaching equilibria. Furthermore, experimental studies in which food web complexity is varied systematically are needed to test the relative importance of theoretical predictions. Greenhouse crops are ideally suited for this latter type of studies, because artificially created communities in biocontrol systems can be manipulated easily. Similarly, greenhouse experiments could give insight into short-term dynamics of interactions for which only equilibrium theory is available. The diversity and complexity of some artificial food webs in greenhouse vegetable crops is presented in the next section.

Food webs in greenhouse crops

The complexities of arthropod communities associated with biocontrol systems varies among crops, because crops differ in susceptibility to pests species and suitability for natural enemies. Here, I present examples of food webs and their interac-

BOX | Aphids

Almost every greenhouse crop is attacked by one or more species of aphids. The green peach aphid, *Myzus persicae* (Sulzer) is the most common species, attacking a wide range of host plants. In this thesis, I used a red phenotype of *M. persicae*, which causes serious damage in sweet pepper (Gillespie et al., 2009). Other important aphids in greenhouse crops are the foxglove aphid *Aulacorthum solani* (Kaltenbach) and the cotton aphid *Aphis gossypii* Glover (Ramakers, 1980; Blümel, 2004). Aphids are phloem feeders and weaken plants by draining their resources and causing severe distortion of growth. Moreover, aphids produce large amounts of honeydew, which can completely cover leaves. This facilitates the growth of sooty mold, which consequently reduces photosynthesis. Aphids reproduce extremely fast (Wyatt & Brown, 1977), which can result in rapid destruction of the crop. Biological control of aphids in greenhouse crops is currently mainly based on releases of the parasitoids *Aphidius colemani* Viereck, *Aphidius ervi* Haliday, *Aphelinus abdominalis* Dalman and the aphidophagous gall midge *Aphidoletes aphidimyza* (Rondani) (Cock et al., 2010, Table 4). Less commonly applied are syrphids, *Episyrphus balteatus* de Geer and chrysopids of the *Chrysoperla carnea*-group, whose larvae are rather specialized aphid predators. The experience is, however, that these predators hardly establish in greenhouse crops as the adults tend to fly away (Ramakers, 1980). Coccinellids are generally recognized as important aphid predators, but hardly used in greenhouses because they also escape. Finally, aphid control may partly be based on generalist predatory bugs which feed on multiple prey, as discussed above.

tions in cucumber and sweet pepper, the two crop systems studied in this thesis.

The most important pests in greenhouse cucumber in Europe and North America are thrips, whiteflies, spider mites and cotton aphids (Shipp, 2004). Modern varieties of greenhouse cucumber are parthenocarpic, so do not produce pollen. This is the reason for generalist predatory bugs not performing well in this crop. The natural enemies used in cucumber are mainly specialized whitefly parasitoids, aphid parasitoids and predatory midges and some specialist and generalist predatory mites (FIGURE 1.2). The interactions in food webs presented in FIGURE 1.2 are based on the literature review presented above.

The second example concerns greenhouse sweet pepper. This is one of the crops where the release of natural enemies for biological control has resulted a complex system of multiple pests and natural enemies, including several different species of generalist predators. These generalists establish easily in this crop, because the plants flower continuously and thus supply nectar and pollen as food for the predators. The most important pests in sweet pepper crops in greenhouses in temperate regions are thrips, spider mites and aphids (Ramakers, 2004), whereas in Mediterranean countries, one of the major pest species is the tobacco whitefly (Calvo et al., 2009). Many other pest species can attack sweet pepper, such as caterpillars of noctuid moths, broad mites, leaf miners and mirid bugs, but they are less important than the pests mentioned above (Ramakers, 2004). Nevertheless, the simultaneous occurrence and need to control these latter pests species results in a complex food web of interacting species (FIGURE 1.3).

The food webs presented in FIGURE 1.2 and 1.3 show that the interactions between a certain pest and its natural enemies are often embedded in a complex web of interactions. For example, intraguild predation is often accompanied by apparent competition between the intraguild prey and several other alternative prey species. Furthermore, the intraguild predators or hyperpredators can also feed on plant-provided food, with the result that plant quality may affect intraguild predation or hyper-

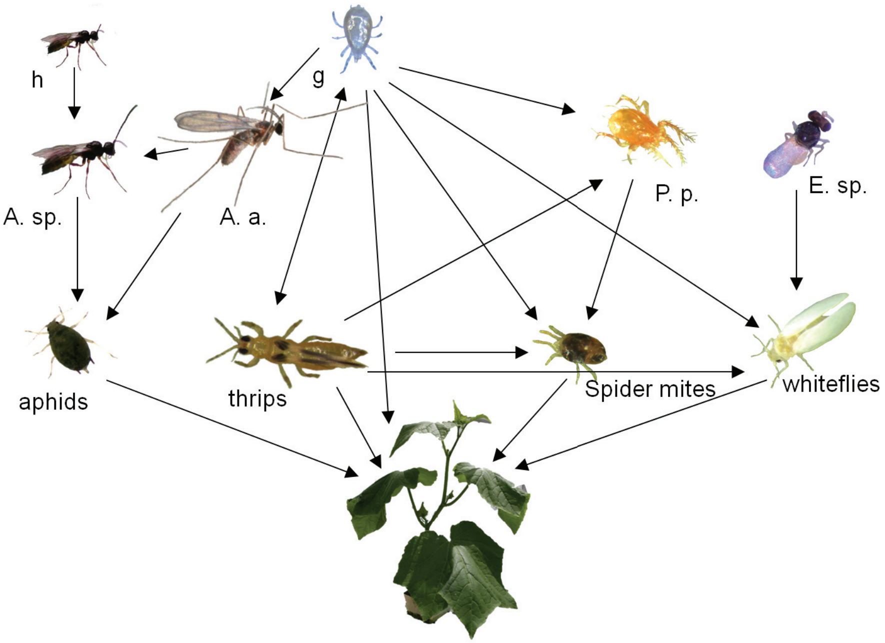

FIGURE 1.2 – A food web of pest species and their most commonly used natural enemies in cucumber. Arrows indicate consumption. Generalist predators are phytoseiid predatory mites (g). Specialist enemies of aphids are parasitoids from the genus *Aphidius* (A. sp.) and the predatory midge *Aphidoletes aphidimyza* (A. a.). Parasitoids are commonly attacked by several species of hyperparasitoids (h). The specialist predator of spider mites is *Phytoseiulus persimilis* (P. p.). Specialist parasitoids of whiteflies are wasps from the genus *Eretmocerus*, or *Encarsia formosa* (E. sp.).

predation (Agrawal & Klein, 2000; Janssen et al., 2003). This illustrates the complexity of biological control, where effects of some interactions may override the effects other interactions (Polis & Strong, 1996). Thus, the study of particular species interactions, such as those between a pest and its natural enemy, should be embedded in empirical studies and models that capture the essence of realistic food webs. Although it may be difficult to disentangle all possible interactions and their importance for biological control, the understanding of such interactions will help in designing effective communities of natural enemies for the suppression of multiple pests. Furthermore, although complex, the artificial food webs of biological control systems are less intricate than most natural systems, and the manipulation of species densities in biological control systems is easier than in natural food webs. Biological control systems therefore offer ideal opportunities for testing food web theory.

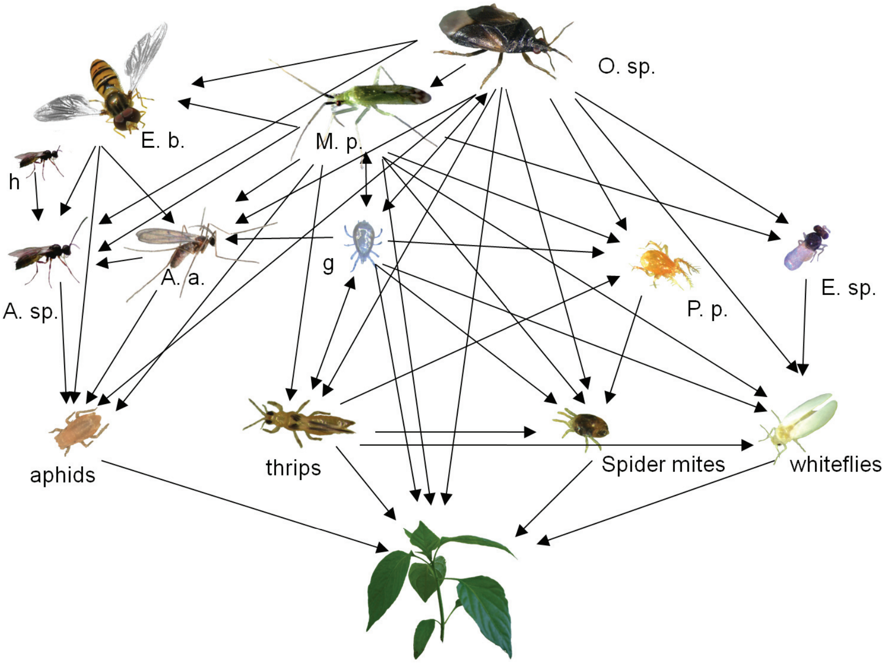

FIGURE 1.3 – A food web of pest species and their most commonly used natural enemies in sweet pepper crops. Arrows indicate consumption. The generalist predators are bugs from the genus *Orius* (O. sp.), the mirid bug *Macrolophus pygmaeus* (M. p.) and generalist phytoseiid predatory mites (g). Specialist enemies of aphids are parasitoids from the genus *Aphidius* (A. sp.), the predatory midge *Aphidoletes aphidimyza* (A. a.) and the syrphid *Episyrphus balteatus* (E. b.). Parasitoids are commonly attacked by several species of hyperparasitoids (h). The specialist predator of spider mites is *Phytoseiulus persimilis* (P. p.). The main whitefly species in sweet pepper is *Bemisia tabaci*, which can be controlled by specialist whitefly parasitoids from the genus *Eretmocerus* (E. sp.).

Outline of this thesis

In this thesis, I study several types of interactions in food webs that occur within arthropod communities on greenhouse crops subject to biological pest control with generalist predators. The central question in all these studies is to which extent patterns expected from food web theory can be identified from the dynamics of arthropod communities in greenhouse crops when using generalist predators, and how interactions in food webs affect the suppression of pest species. In the first part of this thesis, I describe the selection and evaluation of generalist predatory mites for control of thrips and whiteflies in greenhouse cucumbers, and how these predators mediate indirect interactions such as apparent competition and apparent mutualism among thrips, whiteflies and spider mites. In the second part of the thesis, I study the conflicting interactions among predators that are used for biological control of aphids and thrips in sweet pepper, with special emphasis on hyperpredation. In this case, apparent competition occurs between a pest and a natural enemy of another pest. The interactions I studied may serve as tests of general theories on community ecology and contribute to better strategies for multiple pest control in greenhouse crops.

The work described in this thesis started with the selection and evaluation of generalist predators for multiple pest control. I tested several species of predatory mites for the control of thrips in cucumber (CHAPTER 2). The finding of generalist predatory mites that are able to control both thrips and whiteflies gave rise to the question how pest control with a generalist predator works when the two pest species are present simultaneously. In such a case, the two pest species are involved in apparent competition, and, as outlined above, this may have positive or negative effects on the pest densities. In CHAPTER 3, I present the population dynamics of thrips and whiteflies in the presence of their shared predators *Amblyseius swirskii* or *Euseius ovalis.* The indirect predator-mediated interactions between thrips and whiteflies are further studied in CHAPTER 4, where I manipulated the dynamics of whiteflies in such a way, that it would result in positive effects on thrips densities through a shared predator (so-called apparent mutualism). In CHAPTER 5, I increased the food web complexity in cucumber by adding spider mites as a third pest species to the system of thrips, whiteflies and the generalist predator *A. swirskii*. In greenhouse trials, I study the effects of *A. swirskii* on spider mites in the presence and absence of other pest species. In CHAPTER 6, I demonstrate how generalist predatory mites may affect the control of aphids by feeding on the important aphid predator, the gall midge *A. aphidimyza*. CHAPTER 7 shows the effects of generalist predators on the control of aphids and thrips in sweet pepper. Specialist aphid parasitoids and predatory midges were released together with either *N. cucumeris*, a predator of thrips and a hyperpredator of the predatory midge, or *Orius majusculus* (Reuter), a predator of thrips and aphids and intraguild predator of both specialist natural enemies. In CHAPTER 8, I highlight the most important findings of this thesis, discuss these in a broader context of biological control and food web theory and give some ideas and directions for future research.

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Evaluation of phytoseiid predators for control of western flower thrips on greenhouse cucumber

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Ten predatory mite species, all phytoseiids, were evaluated for control of Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), on greenhouse cucumber. This study was done to further improve biological control of thrips on this crop. *Neoseiulus cucumeris* (Oudemans) is at present used for biological control of thrips in greenhouses. Compared to this species, *Typhlodromalus limonicus* (Garman & McGregor), *Amblyseius swirskii* Athias-Henriot and *Euseius ovalis* (Evans) reached much higher population levels resulting in a significantly better control of thrips. *T. limonicus* was clearly the best predator of WFT. Also *Euseius scutalis* (Athias-Henriot) increased to higher populations levels than *N. cucumeris*, but without controlling the thrips, probably because of an unequal distribution of this predator on the plant. *Iphiseius degenerans* (Berlese), *Neoseiulus barkeri* (Hughes), *Euseius finlandicus* (Oudemans) and *Typhlodromus pyri* (Scheuten) did not establish better than *N. cucumeris*. A non-diapausing exotic strain of *N. cucumeris* did not differ from the North European strain. The best performers in this study were all of sub-tropical origin. *T. limonicus*, *A. swirskii* and *E. ovalis* have good potentials for controlling not only thrips but also whiteflies. Factors affecting the efficacy of phytoseiids on greenhouse cucumbers are discussed.

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Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is one of the most serious pest species for greenhouse crops in Europe and North America (Lewis, 1997; Shipp & Ramakers, 2004). Biological control of thrips with phytoseiid mites started with observations of these mites preying on *Thrips tabaci* (Lindeman) in greenhouse crops (MacGill, 1939; Woets, 1973). First attempts to control thrips populations in greenhouses with predatory mites were undertaken with *Neoseiulus barkeri* (Hughes) (= *Amblyseius mckenziei*) (Ramakers, 1980), but the introduction of another indigenous (North European) species, *Neoseiulus cucumeris* (Oudemans), became more successful (de Klerk & Ramakers, 1986). At present, this mite is the most commonly used biological control agent for thrips in various greenhouse crops. Control of thrips with this predator is particularly successful in sweet pepper (Ramakers, 1988). High population levels can be reached in this crop even in absence of thrips, due to the presence of suitable pollen (Ramakers, 1990; van Rijn et al., 1999). Modern greenhouse cucumbers are parthenocarpic and do not produce pollen. For this reason *N. cucumeris* is less successful in greenhouse cucumbers. Several studies show a weak numerical response of *N. cucumeris* at low pest density, resulting in unacceptable high levels of thrips in greenhouse cucumbers before control is achieved eventually (Ramakers et al., 1989; Gillespie, 1989; Brødsgaard & Hansen, 1992). Nevertheless, this mite is widely used because it is the only commercial species available on a large scale. Repeated inundative introductions may provide a reasonable control of WFT (Jacobson et al., 2001), but are not popular because of the high costs involved. Insecticides are still required in order to make a clean start at the beginning of every planting and in summer plantings insecticides rather than predatory mites are used for controlling WFT.

Other phytoseiid predators may provide a more effective control of WFT on cucumber. *N. barkeri*, *Iphiseius degenerans* (Berlese) and *Typhlodromalus limonicus* (Garman & McGregor) were tested in greenhouse cucumbers. *N. barkeri* (Brødsgaard & Hansen, 1992) and *I. degenerans* (van Rijn et al., 1999) did not establish at low thrips densities, whereas *T. limonicus* survived well on cucumbers even with low levels of WFT and gave excellent control of WFT (van Houten, 1996; van Rijn et al., 1999). This mite is not yet available commercially because of mass-rearing difficulties (Mulder et al., 1999). *Iphiseius degenerans* and *N. barkeri* are provided by commercial suppliers, but on a small scale only. *Euseius scutalis* (Athias-Henriot), *Euseius hibisci* (Chant) and *Euseius tularensis* (Congdon) have been evaluated under laboratory conditions in order to improve biological control of WFT (van Houten et al., 1995), but were never tested under greenhouse conditions.

The objective of the present study was to evaluate a number of phytoseiid predators for control of WFT on greenhouse cucumbers in order to select a more effective predator with *N. cucumeris* as a standard. Ten species were selected, based on assumed adaptation to a greenhouse climate, host plant adaptation and feeding behaviour. Of *N. cucumeris* two strains were included, a North European strain and a non-diapausing strain from New Zealand. They are morphologically identical but genetically different (M. Steiner, personal communication).

The ability of predatory mites to survive the sometimes extreme afternoon temperatures in greenhouses is a limiting factor for their establishment (Mori & Chant, 1966; Shipp & van Houten, 1997). Previous work has shown a decline in predatory ability at temperatures above 30°C (Skirvin & Fenlon, 2003) for the tropical species *Phytoseiulus persimilis* Athias-Henriot, but already above 23°C for the North European species *N. cucumeris* (Shipp et al., 1996). Three of the mite species selected for this study were indigenous to The Netherlands, namely *N. cucumeris* (diapausing), *Euseius finlandicus* (Oudemans) and *Typhlodromus pyri* (Scheuten). The others originated from sub-tropical regions: *E. scutalis*, *Euseius ovalis* (Evans), *I. degenerans*, *N. barkeri*, *T. limonicus* and *Amblyseius swirskii* (Athias-Henriot) and *N. cucumeris* (non-diapausing).

Also host plant adaptation will influence establishment of predatory mites on cucumber. Plant architecture, leaf morphology and plant chemistry have been shown to affect the successful establishment of predatory mites (Scott Brown et al., 1999). On *Bryonia dioica* Jacq., the only native Cucurbitaceae in The Netherlands, the phytoseiids *E. finlandicus* and *T. pyri* were most abundant (G. Messelink, unpublished). The subtropical phytoseiids *E. scutalis* and *A. swirskii* were frequently found in a survey on cucumber plants in Egypt (Farrag et al., 1998).

Diet specialization of predatory mites is another important factor that influences the abundance and survival of predatory mites on a particular host plant. A more generalist life style can be useful for predators to survive when only low levels of the target prey are present. All mite species tested in this study are generalist predators. *E. finlandicus* and *T. pyri*, for example, are known to feed on mites, eggs and larvae of insects, on pollen, fungal spores and hyphae, honeydew and plant juice (Zemek & Prenerova, 1997; Abdallah et al., 2001). The degree of specialisation may vary between species. *Euseius finlandicus* appears to be more specialized on pollen feeding than others (McMurtry & Croft, 1997). In this study some additional observations were dedicated to this aspect.

Materials and Methods

Cultures

Ten predatory mite species (TABLE 2.1) were evaluated in three separate greenhouse experiments in 2003. *I. degenerans* was reared on *Ricinus communis* L. in a small greenhouse as described by Ramakers & Voet (1995). All other species were kept in climate rooms, under long-day illumination (L16:D8), at 25°C and 70% RH. *Neoseiulus cucumeris* and *N. barkeri* were reared on *Acarus farris* (Oudemans) and wheat bran (Ramakers & van Lieburg, 1982). The other species were fed with cattail pollen, *Typha latifolia* L. (van Rijn & Tanigoshi, 1999). *Typhlodromalus limonicus* and *T. pyri* were reared on plastic 'arenas' as described by Overmeer (1985), and *E. scutalis*, *E. ovalis*, *E. finlandicus* and *A. swirskii* on sweet pepper leaves (*Capsicum annuum* L. cv. Spirit). WFT was reared on flowering plants of chrysanthemum cv. Miramar. Cucumber plants cv. Aramon were raised in rockwool blocks in a greenhouse compartment.

Greenhouse experiments

Greenhouse experiments were carried out in a greenhouse compartment (18 m^2) on two tables (1 × 3 m) on which cucumbers were cultivated. An ebb-and-flood fertigation system with recirculating nutrient solution was used. In each experiment three

TABLE 2.1 – Origin of predatory mites used in this study.

Species	Plant from which collected	Year of isolation and country
Amblyseius swirskii Athias-Henriot	*Gossypium hirsutum* L.	1997, Israel
Euseius finlandicus (Oudemans)	*Bryonia dioica* Jacq.	2003, The Netherlands
Euseius ovalis (Evans)	*Cucumis sativus* L.	1998, Taiwan
Euseius scutalis (Athias-Henriot)	*Ricinus communis* L.	1998, Jordan
Iphiseius degenerans (Berlese)	*Citrus* sp.	1981, Morocco
Neoseiulus barkeri (Hughes)	*Capsicum annuum* L.	1989, The Netherlands
Neoseiulus cucumeris (Oudemans)	*Capsicum annuum* L.	1981, The Netherlands
N. cucumeris non-diapause strain (nds)	*Thunbergia alata* Bojer	1991, New Zealand
Typhlodromalus limonicus (Garm. & McG.)	*Cyphomandra betacea* Sendt	1996, New Zealand
Typhlodromus pyri (Scheuten)	*Malus sylvestris* Miller	2002, Belgium

TABLE 2.2 – Set-up and climate details of three greenhouse experiments for evaluation of predatory mites for control of *Frankliniella occidentalis* in greenhouse cucumbers.

Experimental details	Experiment 1	Experiment 2	Experiment 3
Mite species	*N. cucumeris* *E. ovalis* *A. swirskii* *T. limonicus*	*N. cucumeris* *N. barkeri* *I. degenerans* *E. scutalis*	*N. cucumeris* *N. cucumeris* nds *E. finlandicus* *T. pyri*
Period	April-May	May-June	August-September
Total duration (days)	31	35	34
Duration with mites (days)	21-22	25	24
Mean temperature (°C)	22.5	23.3	22.6
Temperature range (°C)	18.3-28.0	19.3-28.7	19.1-28.7
Mean RH (%)	72	74	75
RH range (%)	24-93	35-97	34-100

predator species were compared with *N. cucumeris* (TABLE 2.2). Each mite treatment was conducted on a clustered group of five cucumber plants. The young plants were at first-leaf stage when the experiments started and remained on the same rockwool blocks during growth. A distance of 1 m was kept between two groups of five plants on each table. Plants were trained vertically up to a 1.5 m high crop supporting wire. Side shoots were not removed, and once shoots reached the crop supporting wire, they were trained to grow down over this wire. Contamination of treatments was avoided by applying insect glue to the crop supporting wires. Treatments remained isolated by this method, which resulted in only one recorded contamination of a few single female predatory mites of the species *T. limonicus* in the neighbouring treatment of *E. ovalis*. Ten females of *F. occidentalis* were collected from the culture using an aspirator and released on each cucumber plant. Ten days later 10 female predatory mites were introduced per plant on the second leaf. Mites were sampled with a fine paintbrush in the laboratory and placed on leaf discs of sweet pepper (*C. annuum*) (diameter 2 cm) containing cattail pollen. One leaf disc with ten mites was introduced onto each cucumber plant. Temperature and relative humidity were in each experiment comparable with a minimal variation (TABLE 2.2). An assessment of cucumber leaves was conducted at the end of each experiment, 21 to 25 days after releasing the predatory mites (TABLE 2.2). The total number of stem leaves at that time varied between 20 to 25 per plant; including side shoots about 50 leaves per plant. Leaves were collected from each plant at five levels. Stem leaf number 5, 8, 11, 14 and 17, numbered from below, were collected and put separately in plastic bags. In the laboratory leaves were cut in strips of 5 cm wide and assessed under a binocular microscope at 40× magnification. Both sides of the leaves were scanned and numbers of mites and thrips were counted per leaf. Feeding behaviour of these mites was observed during leaf scanning. All mites were mounted in temporary preparations and species and their life-stage were identified under a microscope.

Statistical analysis

The results were analysed with regression analyses, using a generalised linear model (GLM) accounting for a Poisson distribution of the data (McCullagh & Nelder, 1989). Plants with the same treatment were spatially clustered rather than fully randomised in order to avoid mixing of predatory mite species. Observations per plant were assumed to be independent, though without any blocking structure. Differences between treatments were tested against 5% level of significance using the estimations of the differences and their standard errors on the link scale (Lane & Nelder, 1982).

Results

At the end of the first experiment, the total number of mites, including eggs and thrips larvae per five leaves differed statistically significantly for each species of predatory mites (TABLE 2.3). *T. limonicus* reached the highest population levels, followed by *A. swirskii*, *E. ovalis* and *N. cucumeris*. Predatory mite densities were inversely related to thrips densities in all cases. Predatory mites were recorded on all sampled leaves (FIGURE 2.1). *N. cucumeris* had the lowest relative abundance compared to the other species. Thrips larvae were equally distributed and present on all leaves on plants with *N. cucumeris* and *A. swirskii* (FIGURE 2.2). On plants with *T. limonicus* thrips lar-

TABLE 2.3 – Mean (± SE) numbers of predatory mites (including eggs) and thrips larvae per five leaves of cucumber plant at the end of experiment 1.

Species	Predatory mites/plant	Thrips larvae/plant
N. cucumeris	6.8 ± 2.2 a	140.4 ± 29.6 d
E. ovalis	28.6 ± 6.9 b	116.6 ± 30.8 c
A. swirskii	61.0 ± 9.1 c	27.4 ± 8.8 b
T. limonicus	86.4 ± 13.6 d	0.8 ± 0.4 a

Means within a column followed by different letters are significantly different (p<0.05).

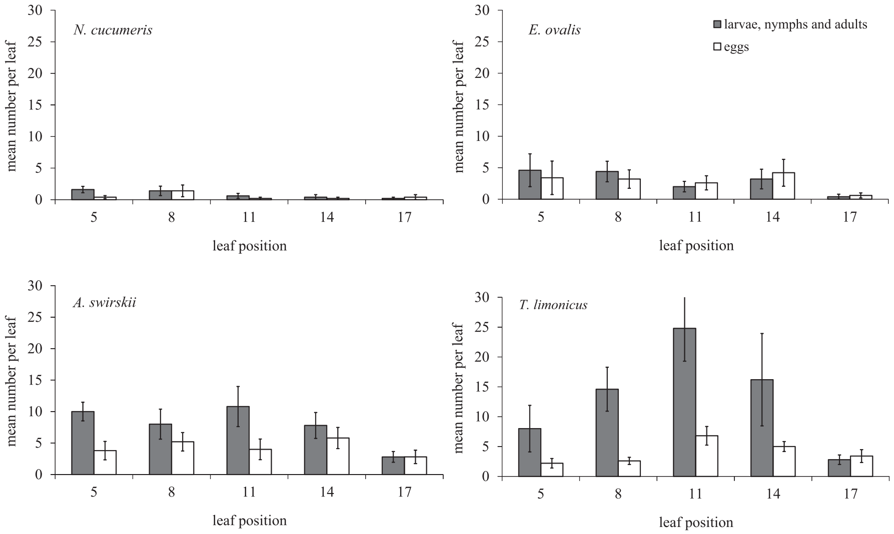

FIGURE 2.1 – Distribution of predatory mites (means ± SE) in cucumber plants at the end of experiment 1. Leaves are numbered from below.

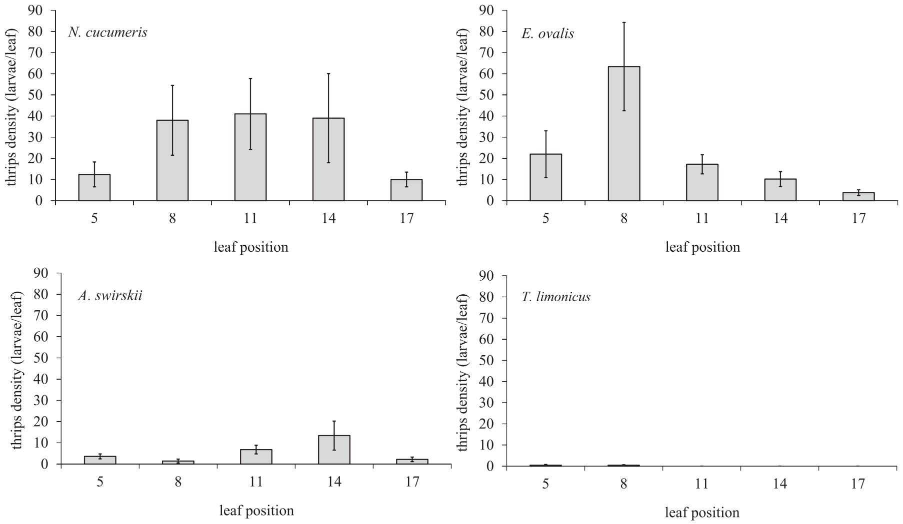

FIGURE 2.2 – Distribution of thrips larvae (means ± SE) in cucumber plants at four mite treatments at the end of experiment 1. Leaves are numbered from below.

vae were recorded in very low numbers on lower leaves. On plants with *E. ovalis* the highest thrips densities were recorded on the lower leaf number 8 (FIGURE 2.2).

At the end of the second experiment the predatory mite *E. scutalis* reached the highest populations levels, but thrips levels were also the highest (TABLE 2.4). The mites *N. cucumeris* and *I. degenerans* had similar population levels, while *N. barkeri* had the lowest densities (TABLE 2.4) with very low numbers of eggs (FIGURE 2.3). Thrips densities were the lowest with *N. barkeri* and *I. degenerans* (TABLE 2.4). *E. scutalis* was abundant on the lowest leaves (FIGURE 2.3), whereas thrips larvae were abundant on the upper leaves of these plants (FIGURE 2.4). *I. degenerans* was also most abundant on the lower leaves (FIGURE 2.3), but thrips larvae were equally distributed. In the two remaining mite treatments *N. cucumeris* and *N. barkeri*, both mites and thrips larvae were equally distributed on the plants (FIGURES 2.3 and 2.4).

At the end of the third experiment the numbers of predatory mites remained low in all treatments and no significant differences were found among the mite species (TABLE 2.5). The mite *T. pyri* disappeared completely. Consequently, thrips population was highest in this treatment. Population levels of thrips larvae were not different between the two strains of *N. cucumeris*, whereas *E. finlandicus* had the lower thrips densities (TABLE 2.5). Overall density of predators in this experiment was too low to collect information about spatial distribution. Distribution of mites and thrips larvae on the plants from this experiment is not shown because of low mite numbers.

Sex ratios based on the total number of adult mites from the assessed leaves, differed by mite species. *I. degenerans*, *N. cucumeris* and *E. scutalis* had relatively high

TABLE 2.4 – Mean (± SE) numbers of predatory mites (including eggs) and thrips larvae per five leaves of cucumber plant at the end of experiment 2.

Species	Predatory mites/plant	Thrips larvae/plant
N. cucumeris	23.6 ± 5.9 b	159.2 ± 39.1 b
N. barkeri	16.3 ± 1.4 a	137.5 ± 45.2 a
I. degenerans	27.5 ± 7.2 b	139.0 ± 23.3 a
E. scutalis	55.8 ± 6.0 c	300.8 ± 54.9 c

Means within a column followed by different letters are significantly different (p<0.05).

TABLE 2.5 – Mean (± SE) numbers of predatory mites (including eggs) and thrips larvae per five leaves of cucumber plant at the end of experiment 3.

Species	Predatory mites/plant	Thrips larvae/plant
N. cucumeris	2.4 ± 0.7 a	112.4 ± 24.9 b
N. cucumeris nds	1.2 ± 0.6 a	113.8 ± 30.0 b
E. finlandicus	1.2 ± 0.6 a	70.6 ± 17.9 a
T. pyri	0.0 ± 0.0 a	197.6 ± 51.5 c

Means within a column followed by different letters are significantly different (p<0.05).

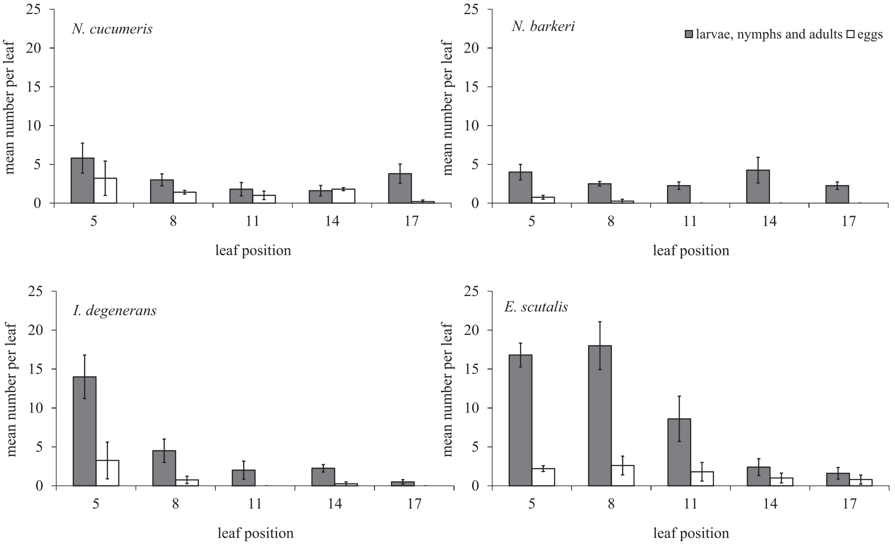

FIGURE 2.3 – Distribution of predatory mites (means ± SE) in cucumber plants at the end of experiment 2. Leaves are numbered from below.

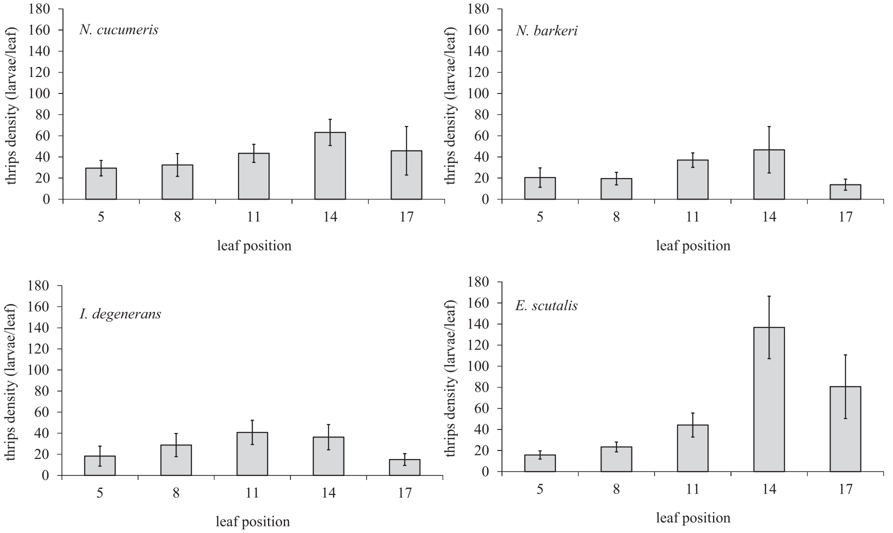

FIGURE 2.4 – Distribution of thrips larvae (means ± SE) in cucumber plants at four mite treatments at the end of experiment 2. Leaves are numbered from below.

TABLE 2.6 – Comparative distribution of life stages of predatory mite species (percentages) and their sex ratios from the total number of collected cucumber leaves in all three experiments.

Species	Egg	Larva	Nymph	Male	Female	Female with egg[4]	Sex ratio (% females)	N
I. degenerans[1]	15	5	25	25	22	7	54	110
N. cucumeris[2]	31	4	27	15	16	5	59	164
E. scutalis[3]	15	14	18	18	25	9	66	271
T. limonicus[3]	23	18	28	6	18	7	79	433
A. swirskii[3]	35	11	27	5	17	5	83	306
N. barkeri[1]	6	3	24	9	39	18	86	66
E. ovalis[3]	49	14	15	2	13	8	91	143

[1] Total number of 20 cucumber leaves from one experiment (loss of one plant).

[2] Total number of 75 cucumber leaves from three experiments.

[3] Total number of 25 cucumber leaves from one experiment.

[4] Visible under microscope.

numbers of males compared to other species (TABLE 2.6). Final populations of *I. degenerans*, *N. cucumeris*, *E. scutalis*, *T. limonicus*, *A. swirskii* and *E. ovalis* all consisted of more than 46% of immature and egg stages, whereas these stages were present in relatively low numbers in the population of *N. barkeri*, 33% (TABLE 2.6). *Typhlodromalus pyri* and *E. finlandicus* were excluded from this analysis because of the low numbers found at the end of the experiment.

Discussion

Typhlodromalus limonicus was clearly the best predator of WFT in greenhouse cucumber, confirming earlier experiments (van Houten, 1996; van Rijn et al., 1999). *Typhlodromalus limonicus*, *A. swirskii* and *E. ovalis* reached both significantly higher population levels and gave a significantly better control of thrips than the standard species *N. cucumeris.*

Euseius scutalis increased to higher populations levels than *N. cucumeris*, but thrips levels were also high. High densities of this predator were present on the lower leaves, whereas thrips reached high densities on the upper leaves in this treatment. Thrips control was insufficient for *E. scutalis*, probably because of this unequal distribution. However, it should be mentioned that this situation only represents one moment, 25 days after introduction of the predatory mites. It may be possible that at a later moment this predator, due to its high number, is able to suppress the thrips population. Interacting populations of predator and prey often show strong fluctuations initially, which means that the result depends on the moment of sampling (van Rijn et al., 2002). A possible mechanism responsible for the unequal distribution of *E. scutalis*, might be a mutual avoiding behaviour, since thrips feed on mite eggs (Faraji et al., 2002). A second mechanism might be that the speed of predator response to prey fluctuations differs among predator species, due to differences in predation rate, development and reproduction.

Iphiseius degenerans also preferred the lower leaves (FIGURE 2.2), but that did not result in higher numbers of thrips larvae. *Iphiseius degenerans* may still provide reasonable thrips control since this mite is relatively large and may be more successful in capturing thrips larvae. The species *I. degenerans*, *N. barkeri*, *E. finlandicus* and *T. pyri* did not establish better than *N. cucumeris* on cucumber.

The best performers in this study were all sub-tropical, probably better adapted to the greenhouse climate. Host plant characteristics such as plant architecture, leaf morphology, leaf physiology or microclimate, may play a role for the establishment of predatory mites on cucumber. Observations under the microscope did not suggest any physical disruption by plant hairs, on the contrary, hairs were often used for attaching eggs. The indigenous species *E. finlandicus* and *T. pyri* were abundant on a wild cucurbit, but did not establish better on cucumber than *N. cucumeris* did.

Final populations of *I. degenerans*, *N. cucumeris*, *E. scutalis*, *T. limonicus*, *A. swirskii* and *E. ovalis* all consisted of minimally 46% of immature and egg stages. This indicates that abundant food was present for these predatory mites (Kreiter et al., 2002). The ability of immature predators to seize thrips larvae upon attack differs between phytoseiid species (Sabelis & van Rijn, 1997). Such differences may produce different life stage distributions and might explain the relatively low numbers of immature stages in the population of *N. barkeri*, since this species is relatively small compared to the other tested phytoseiids.

On greenhouse crops, alternative food may be scarce. Moreover modern greenhouse cucumber varieties do not produce pollen. The ability to feed on additional food sources like plant tissue, nectar or fungal spores might improve establishment of predatory mites. A recent study suggested that *E. scutalis* feeds on plant tissue of cucumber unlike *A. swirskii*, based on their higher mortalities on cucumber leaves treated with a systemic insecticide (Nomikou et al., 2003). It is observed that *T. limonicus* often has green gut content, suggesting that this predator also feeds on plant tissue (G. Messelink, personal observations).

The mites tested in this study are all generalist predators and able to feed on various insects and mites, however the prey suitability of thrips for these mites might differ per species. *Neoseiulus cucumeris* was described before as *Typhlodromus thripsi* (MacGill, 1939), but other authors associated this predator with spider mites (Nesbitt, 1951). References about *A. swirskii*, *E. ovalis* and *E. scutalis* as thrips predators are scarce. *A. swirskii* was reported feeding on *T. tabaci* (Hoda et al., 1986) and *Retithrips syriacus* (Mayet) (Swirski et al., 1967). *Euseius ovalis* was mentioned as a predator of the thrips species *Scirtothrips dorsalis* (Hood) (Manjunatha et al., 2001) and *E. scutalis* was noted to feed on *F. occidentalis* (van Houten et al., 1995), *Scirtothrips citri* (Moulton) (Bonfour & McMurtry, 1987) and *R. syriacus* (Swirski et al., 1967). Direct observations of these mites under a binocular microscope also showed feeding on thrips larvae (this study). Other prey was not present in the current study.

Prey suitability of WFT for predatory mites can be determined by measuring rates of predation and oviposition on a diet of *F. occidentalis* larvae. Van Houten et al. (1995) showed in a study with seven phytoseiid species that *T. limonicus* had the highest predation rate (6.9 larvae/female/day) and the highest oviposition rate (3.2 eggs/day) on a diet of first instar *F. occidentalis* larvae. *N. cucumeris* was second best with a predation rate of 6.0 larvae/female/day and an oviposition rate of 2.2 eggs/day. *E. scutalis* showed a much lower predation rate (1.3 larvae/female/day) and oviposition rate (0.3 eggs/day). In our experiment though, *E. scutalis* reached a much higher population level than *N. cucumeris*. These results show that predation rates on plants with thrips can differ from a laboratory situation where larvae of thrips are offered on leaf discs. Predation and oviposition rate are just part of a number of factors that determine the success or failure of phytoseiid predators in a greenhouse situation. Observations under the microscope showed a higher searching activity of *T. limonicus*, *A. swirskii*, *E. scutalis* and *E. ovalis* than *N. cucumeris*, that could be more typified as a 'sit and wait predator'. Higher searching rates will possibly result in more encounters and higher predation rates.

Amblyseius swirskii and *E. scutalis* have recently shown to be able to suppress populations of *B. tabaci* on cucumber plants (Nomikou et al., 2002). Feeding and reproduction on *B. tabaci* were also observed for the species *T. limonicus* (Swirski & Dorzia, 1969) and *E. ovalis* (Borah & Rai, 1989). Thus, it is possible that one species of predatory mite can suppress populations of both thrips and whiteflies, the two main pest problems in greenhouse cucumbers. Control of whiteflies with *A. swirskii* on cucumber was much better when pollen was added, because of the higher numbers of predators on leaves with pollen (Nomikou, 2003). WFT and whiteflies are present most of the time in a greenhouse situation, at least in low numbers. Predators that feed on thrips and whiteflies will probably establish better and reach higher numbers that might result in better control of both pests.

The predatory mites *T. limonicus*, *A. swirskii* and *E. ovalis* seem to have the best potential for biological control of thrips and possibly whiteflies in greenhouse cucumbers. Commercial availability of these mites will depend on the interest of producers of natural enemies and the costs related to mass production of these species.

Acknowledgements

This study was supported by the Dutch Product Board for Horticulture. We thank Maria Nomikou (University of Amsterdam) for providing *E. ovalis*, *E. scutalis* and *A.*

swirskii and Jan Vermeulen (Biobest NV) for providing *T. pyri* to start new cultures. J. Klap (Applied Plant Research) is acknowledged for statistical support.

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Biological control of thrips and whiteflies by a shared predator: Two pests are better than one

G.J. Messelink, R. van Maanen, S.E.F. van Steenpaal & A. Janssen

We studied the capacity of one species of predator to control two major pests of greenhouse crops, Western flower thrips [*Frankliniella occidentalis* (Pergande)] and the greenhouse whitefly [*Trialeurodes vaporariorum* (Westwood)]. In such a one-predator-two-prey system, indirect interactions can occur between the two pest species, such as apparent competition and apparent mutualism. Whereas apparent competition is desired because it brings pest levels down, apparent mutualism is not, because it does the opposite. Because apparent competition and apparent mutualism occurs at different time scales, it is important to investigate the effects of a shared natural enemy on biological control on a time scale relevant for crop growth. We evaluated the control efficacy of the predatory mites *Amblyseius swirskii* Athias-Henriot and *Euseius ovalis* (Evans) in cucumber crops in greenhouse compartments with only thrips, only whiteflies or both herbivorous insects together. Each of the two predators controlled thrips, but *A. swirskii* reduced thrips densities the most. There was no effect of the presence of whiteflies on thrips densities. Whitefly control by each of the two predators in absence of thrips was not sufficient, yet better with *E. ovalis*. However, whitefly densities in presence of thrips were reduced dramatically, especially by *A. swirskii*. The densities of predators were up to 15× higher in presence of both pests than in the single-pest treatments. Laboratory experiments with *A. swirskii* suggest that this is due to a higher juvenile survival and developmental rate on a mixed diet. Hence, better control may be achieved not only because of apparent competition, but also through a positive effect of mixed diets on predator population growth. This latter phenomenon deserves more attention in experimental and theoretical work on biological control and apparent competition.

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The use of different natural enemies for the biological control of different pest species results in the creation of complex artificial food webs in agricultural crops. This implies that pest densities are not only determined by the natural enemies of that pest, but also by direct and indirect interactions with other pests and enemies, and such interactions can affect biological control (Rosenheim et al., 1995; Janssen et al., 1998). The use of one natural enemy to control several pests will result in food webs simpler than those in which different enemies are introduced against each pest species. However, such a natural enemy may mediate indirect interactions between these pest species, which in turn may be beneficial or detrimental to biological control. One such interaction between two pest species through a shared natural enemy is apparent competition, which Holt (1977, 1984) defined as the indirect interaction between prey through shared predation. When two prey species share a natural enemy, the equilibrium density of one of the prey species decreases with increasing equilibrium density of the other species. This is because the density of the shared natural enemy increases with the increased equilibrium density of either prey species (Holt, 1977; Müller & Godfray, 1997; Janssen et al., 1998; van Rijn et al., 2002; Morris et al., 2004). This interaction can even lead to exclusion of one of the two prey species (Bonsall & Hassell, 1997).

The addition of alternative food to better suppress a pest species through apparent competition has been used in biological control, often with the desired result (Karban et al., 1994; Hanna et al., 1997; van Rijn et al., 2002; Liu et al., 2006). However, the alternative food often consists of non-prey, such as pollen (van Rijn et al., 2002), or the alternative prey is not a pest, but serves primarily as alternative food to build up predator populations (Karban et al., 1994; Hanna et al., 1997; Liu et al., 2006). Here, we investigate the effects of apparent competition between two prey species that are both serious pests of various crops worldwide.

Two prey species that share a predator may also affect each other's densities positively because an increase in the numbers of one pest species may lead to predator satiation, resulting in decreased predation on the other pest species. In addition, predators may switch to the most abundant prey species, thus releasing the other species from predation. Such positive indirect effects are referred to as apparent mutualism (Holt & Lawton, 1994; Abrams & Matsuda, 1996). Apparent mutualism occurs mostly at a shorter time scale than apparent competition (Harmon & Andow, 2004; van Veen et al., 2006). Hence, depending on time-scale and prey preference, a natural enemy that feeds on two pest species can mediate mutualistic or antagonistic interactions between the two pests. The use of one species of natural enemy for biological control of two pests may thus result in reduced control in the short term, but increased control in the long term (van Rijn et al., 2002). It is therefore relevant to assess the time scale at which indirect interactions occur. In our system, this scale is set by the length of the growing season of the greenhouse crop.

In the literature on apparent competition, little attention has been given to the effect of mixed diets on the performance of predators. Mixed diets are known to have positive effects on reproduction in some predator species (Wallin et al., 1992; Toft, 1995; Evans et al., 1999), and the effect of adding a new prey species would then surpass that of simply adding more prey items with the same nutritive value. Therefore, we also investigated the effects of a mixed diet on predator survival and reproduction.

The experimental system

Western flower thrips [*Frankliniella occidentalis* (Pergande)] and greenhouse whitefly [*Trialeurodes vaporariorum* (Westwood)] are two major pest species in various crops in Northern Europe and North America (Lewis, 1997; Byrne et al., 1990). The phytoseiid *Amblyseius swirskii* Athias-Henriot (Chant & McMurtry, 2004) has recently been shown capable of suppressing populations of the tobacco whitefly [*Bemisia tabaci* (Gennadius)] (Nomikou et al., 2001, 2002). *Euseius ovalis* (Evans), another species of predatory mite, also feeds and reproduces on a diet of *B. tabaci* (Borah & Rai, 1989). An evaluation of phytoseiids for control of Western flower thrips in greenhouse cucumber showed that *A. swirskii* and *E. ovalis*, amongst others, are much more effective thrips predators than *Neoseiulus cucumeris* (Oudemans), a phytoseiid that is often used to control thrips, (Messelink et al., 2005, 2006). Thus, each of the two mites *A. swirskii* and *E. ovalis* can potentially control whiteflies and thrips.

We studied the dynamics of Western flower thrips and greenhouse whiteflies separately as well as together on cucumber plants (cv. Aviance RZ) with one of the two predatory mite species in small greenhouse compartments. Cucumber plants have a short cropping season, and short-term effects of shared predation, such as apparent mutualism, may determine the dynamics of the pests and predators. For biological control, it is therefore important to assess whether the effects of shared predation on pest levels are positive or negative. In order to detect an effect of mixed diet on the population dynamics of the predator, we also compared the effect of diets consisting of pest species separately or of a mix of both species on several life-history parameters of *A. swirskii* that are important for population dynamics (oviposition, juvenile survival, development).

Materials and Methods

Cultures

For the greenhouse experiments, the predatory mites were reared on flowering *Ricinus communis* L. plants in small greenhouses. The predators fed on the pollen, amply produced by these plants. Western flower thrips were reared on flowering chrysanthemum plants cv. Miramar. The greenhouse whitefly was reared on tobacco plants (*Nicotiana tabacum* L.). Cucumber plants cv. Aviance RZ were grown in rock wool blocks in a greenhouse compartment without any application of pesticides. The greenhouse experiments were carried out at Wageningen UR Greenhouse Horticulture, Naaldwijk, The Netherlands.

For the laboratory experiments, *A. swirskii* mites were reared on plastic arenas (8 × 15 cm), placed on a wet sponge in a plastic tray containing water (Nomikou et al., 2003a). They were fed cattail pollen, *Typha latifolia* L. twice per week. Western flower thrips were reared in climate boxes and greenhouse whiteflies in a walk-in climate room. Their host plants were cucumber plants cv. Aviance RZ, grown from seeds in plastic pots (2 l) with soil and kept in a walk-in climate room, free of herbivores, before use in the arthropod cultures. Laboratory experiments were carried out at the section Population Biology, University of Amsterdam, The Netherlands.

Greenhouse experiments

The effects of the predators *A. swirskii* and *E. ovalis* on Western flower thrips, greenhouse whitefly and a combination of these two pests was examined in a greenhouse experiment in twelve separate compartments (each 18 m^2) that each contained two tables (1 × 3 m) on which cucumber plants were grown. The experiment was set-up as a split-plot experiment with four replicates. Each block contained one compartment with thrips, one compartment with whiteflies and one compartment with thrips and whiteflies. In each compartment, *A. swirskii* was released on plants on one table and *E. ovalis* on the other table. Note that we did not include control treatments in which the pest species had no predators. Hence, we have no data on the effects of the pest species on each other through the shared host plant. The plants in the treatments with both pest species had such low damage levels that exploitative competition between the two pests was improbable, certainly when we consider the large leaf size of a cucumber crop (in this treatment 250-450 cm^2). However, the two pests possibly affected each other through induced plant responses (Karban & Carey, 1984). This will be the subject of forthcoming research. Secondly, it should be realized that the predator treatments are strictly not independent, because thrips and whiteflies were able to migrate between the two tables in a compartment. This migration might result in an underestimate of the control capacity of the best performing predator, and an overestimate of the capacity of the other predator. However, for analyzing results we assumed the predator treatments to be statistically independent.

Each greenhouse compartment had a small closed entrance corridor without windows and was equipped with an air pressure system in order to minimize contamination with organisms from outside. Plants were at the fifth-leaf stage when the experiments started, and roots were preventively treated with Propamocarb against *Pythium* spp. Four plants were placed on two pieces of a rock wool substrate slab on each table. The experiment started in week number 12. The rock wool slabs were continuously immersed in a nutrient solution that was automatically supplied once per day. Plants were cultivated vertically up to a 1.5 m high crop supporting wire. Side-shoots were removed until the top of the plant reached the crop supporting wire. Later on, plant shoots and side shoots grew down over the crop supporting wire. Contamination of treatments was avoided by applying insect glue to the wires supporting the crop and by keeping the plants isolated in the water layer on the tables.

Pests were introduced one day after the cucumber plants were planted. Forty adult female thrips, collected from the culture using an aspirator, were released on each table. A total of 120 adult whiteflies were released per table. The population of whiteflies contained on average 42% females. The same numbers were released in the treatment with both pests. Predatory mites were released seven days after introducing the pests. Female predatory mites were sampled with a fine paintbrush in the laboratory and placed on leaf discs of sweet pepper (*Capsicum annuum*) (diameter 2 cm) containing cattail pollen. One leaf disc with fifteen mites was introduced on the upper leaves of each cucumber plant.

The experiment lasted 11 weeks. During this period, the crop was inspected twice a week, and cucumbers were harvested as soon as they reached the standard fruit size. Different treatments were handled by different persons to avoid cross-contamination. The treatments with different pests were indeed not invaded by other pests, except for one compartment with whiteflies, which was invaded by spider mites in week 8. This spot was treated by releasing 100 adults of the predatory mite *Phytoseiulus persimilis* Athias-Henriot, resulting in successful control. *Phytoseiulus persimilis* does not consume thrips or whiteflies, and they were not observed after the spider mites had been eradicated, within a few days. Powdery mildew occurred occasionally during the experiment, but infections remained limited to small spots because the cucumber variety used is partially resistant to mildew.

The numbers of predatory mites and pests were assessed 4, 6, 8 and 10 weeks after introducing the predatory mites. Plants were not sampled during the first 4 weeks in order not to cause disturbance. Assessing the populations was done by collecting six leaves from each treatment by randomly choosing three shoots of which the sixth and eighth leaf from the tip was collected. These leaves were each put in a separate plastic bag and transported to the laboratory where they were cut into strips of 5 cm. The number of mites, thrips and whiteflies were counted on both sides of the leaves using a binocular microscope (40×). All mites were slide-mounted for identification to species, gender and life-stage under a microscope (400×). Only the larval stages of thrips were counted, and eggs, larvae and pupae of whiteflies were counted separately. When densities of whiteflies exceeded 500 individuals per leaf, densities were assessed on part of each leaf only, and extrapolated to the whole leaf. The leaf area of each collected leaf was measured with an optical area meter (LI-COR LI-3100) after mite and pest densities were quantified. The average temperature and relative humidity were comparable for each block treatment (TABLE 3.1).

The results were analysed with linear mixed effects models (lme in R), with time as random factor nested in blocks to correct for pseudoreplication due to repeated measures (Crawley, 2002). The numbers of thrips and whiteflies were log (x+1) trans-

TABLE 3.1 – Experimental conditions in the four experimental blocks during the greenhouse experiment. Each block contained three separate greenhouses with a thrips, whitefly or thrips & whitefly treatment.

	Block			
	1	2	3	4
Mean temperature (°C)	22.5	22.2	22.8	22.5
Mean relative humidity (%)	74	75.8	76.7	80.5

formed before the analysis, in order to stabilise variance. Treatments were compared through model simplification by combining treatments (Crawley, 2002).

Oviposition, juvenile survival and development, and predation

The oviposition rate and predation rate of *A. swirskii* was measured during 3 days on three different diets: whitefly eggs, young first instars thrips and a mixture of whitefly eggs and young first instar thrips. These stages are most vulnerable to these predators (Nomikou et al., 2004; R. van Maanen, pers. obs.). Adult female predators (8-11 days old since egg stage) were tested individually on a leaf disc (24 mm diameter) with one of the three different diets. The numbers of whitefly eggs ranged from 21 to 59 eggs/leaf disc and the numbers of young first instars thrips were 8 or 15/leaf disc. Previous predation tests showed that these densities are high enough to ensure maximum prey consumption (Nomikou et al., 2002). Cohorts of whitefly eggs were prepared in advance (Nomikou et al., 2003b) and first instar thrips larvae were reared on cucumber leaves with cattail pollen placed on wet cotton wool in Petri dishes. The numbers of both thrips and whiteflies in the mixed diet were equal to the single prey diets, hence, supplied additively. Predation was recorded as the number of whitefly eggs consumed (as judged by the transparent cuticle) and first-instar thrips consumed (as judged by the remains) after 24 and 48 h. Because oviposition rates are affected by the previous food source of the adult predatory mites (Sabelis, 1990), we discarded data from the second day of the experiment only (Sabelis, 1986). Oviposition was compared between days and diets using a generalized linear mixed effects model with Poisson errors and a random factor within replicates to correct for pseudoreplication, using R (lmer; R Development Core Team, 2005). Predation was analysed for thrips larvae and whitefly eggs separately with generalized linear models with Poisson error distributions and diet as factor.

For the effects of diet on juvenile survival and development, we placed between 80 and 100 female mites from the culture on a plastic arena with cattail pollen. After less than 24 h, we transferred their eggs to clean cucumber leaf discs, each egg on a separate disc. We transferred the larva, directly after emerging, to a leaf disc with whitefly eggs, young first-instar thrips or a mixture of whitefly eggs and young first-instar thrips. Every other day, mites were transferred to a new leaf disc with whitefly eggs, young first-instar larvae or the two prey together. Survival and stage of the predator were recorded daily until the mites reached adulthood. Kaplan-Meier survival curves on different diets were fitted and compared using the log-rank test (Hosmer & Lemeshow, 1999, library survival, R Development Core Team, 2005). Juvenile development was analysed using a time-to-event analysis with log-rank tests. Three replicates were performed with 45, 45 and 37 individuals, the first 2 with 15 individuals per diet, the last with 8 individuals on a mixed diet, 15 on a thrips diet and 14 on a diet of whiteflies.

Results

Greenhouse experiments

Thrips

There was a clear effect of treatment on thrips densities (Figure 3.1, lme: $F_{3,377}$ = 124.8, p<0.0001). *Amblyseius swirskii* reduced thrips to very low densities (Figure 3.1A). The highest thrips densities were observed at the first assessment after 4 weeks, after which they went down to less than one larva per leaf in the following weeks (Figure 3.1A). *Euseius ovalis* was less successful in controlling thrips; the ultimate density was between 80 and 20 larvae per leaf (Figure 3.1B, difference between *A. swirskii* and *E. ovalis*: model simplification after linear mixed effects model (lme), thrips only: log likelihood ratio (LR) = 167.1, d.f. = 8,7, p<0.0001, thrips plus whitefly: LR = 140.1, d.f. = 8,7, p<0.0001). This result is qualitatively similar to earlier experiments in which ultimate thrips densities were 4× higher in the presence of *E. ovalis* than in the presence of *A. swirskii* (Messelink et al., 2006). In the treatments with the two pest species present, densities of thrips were not affected by the presence of whiteflies, irrespective of whether *A. swirskii* or *E. ovalis* was the shared predator (Figure 3.1A,B; *A. swirskii*: LR = 0.019, d.f. = 8,7, p = 0.89, *E. ovalis*: LR = 2.17, d.f. = 6,7, p = 0.14).

Whiteflies

There was a significant effect of treatment on densities of whiteflies (Figure 3.2; $F_{3,377}$ = 69.2, p<0.0001), but neither of the two predators could prevent an increase in the populations of whiteflies, resulting in thousands of immature whiteflies per leaf (Figure 3.2A,B). However, the predators delayed the population increase of whiteflies considerably compared to that expected under exponential growth and to that observed in earlier experiments without predatory mites (Messelink, pers. obs.). *Euseius ovalis* was more successful in controlling whiteflies than *A. swirskii* when thrips were absent (Figure 3.2A,B; LR = 64.1, d.f. = 8,7, p<0.0001).

In the treatments with both thrips and whiteflies, the two predators reduced densities of whiteflies clearly more than in the treatments without thrips (Figure 3.2A,B; *A. swirskii*: LR = 113.1, d.f. = 8,7, p<0.0001; *E. ovalis*: LR = 24.2, d.f. = 8.7., p<0.0001). *Amblyseius swirskii* reduced whitefly densities more than *E. ovalis* (Figure 3.2A,B; LR

= 146.0, d.f. = 8,7, p <0.0001). In the case of *A. swirskii*, whiteflies went practically extinct (FIGURE 3.2A), and with *E. ovalis*, densities of whiteflies went down from a peak of approximately 1000 immatures per leaf in the sixth week, to approximately 500 immatures per leaf in the tenth week (FIGURE 3.1B). This suggests an indirect interaction between thrips and whiteflies mediated by the shared predator (apparent competion) or via the host plant.

Predators

There was a significant effect of the pest species on the densities of predators (lme: *A. swirskii*: $F_{2,282} = 151.0$, p<0.0001; *E. ovalis*: $F_{2,282} = 131.1$, p<0.0001). Compared

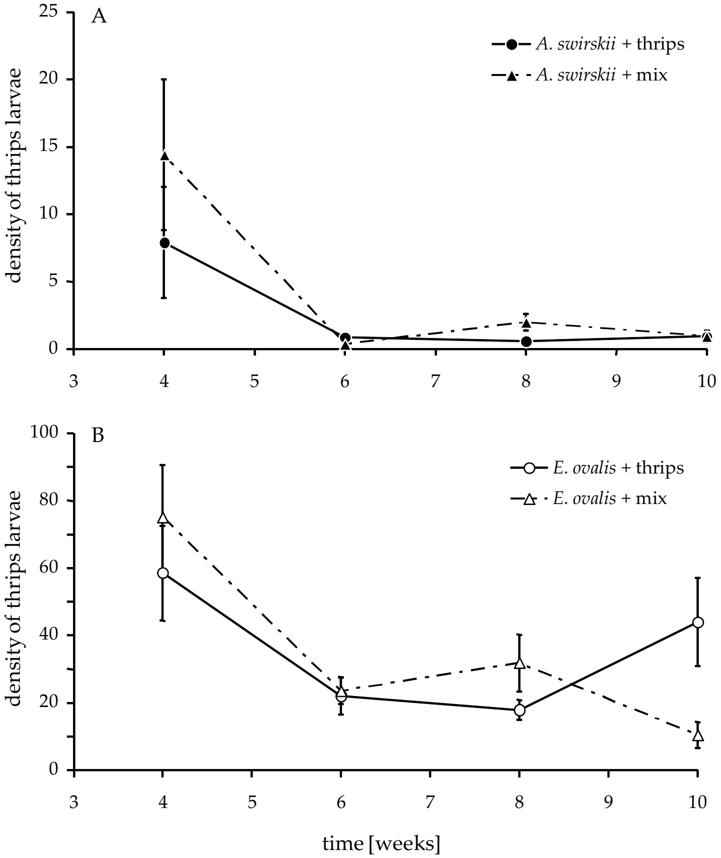

FIGURE 3.1 – The dynamics of Western flower thrips during a 10-week greenhouse experiment in presence of the predatory mites *A. swirskii* (A) and *E. ovalis* (B). Shown are average densities (± SE) of thrips larvae in the presence (triangles) or absence (circles) of greenhouse whiteflies.

to the treatments involving single pest species, the presence of thrips and whiteflies together resulted in higher densities of *A. swirskii* (FIGURE 3.3A; thrips vs. mix: LR = 128.2, d.f. = 7,6, p<0.0001; whitefly vs. mix: LR = 189.5, d.f. = 7,6, p<0.0001), but the difference in densities between treatments with single pest species was also significant (FIGURE 3.3A; thrips vs. whitefly: LR = 9.3, d.f. = 7,6, p = 0.0023). The same was found for *E. ovalis*, (FIGURE 3.3B; thrips vs. mix: LR = 126.3, d.f. = 7,6, p<0.0001; whitefly vs. mix: LR = 168.4, d.f. = 7,6, p<0.0001, thrips vs. whitefly: LR = 6.4, d.f. = 7,6, p = 0.011). Together with the lower densities of whiteflies in the treatments with both pests, these findings are suggestive of apparent competition between thrips and whiteflies on whitefly densities.

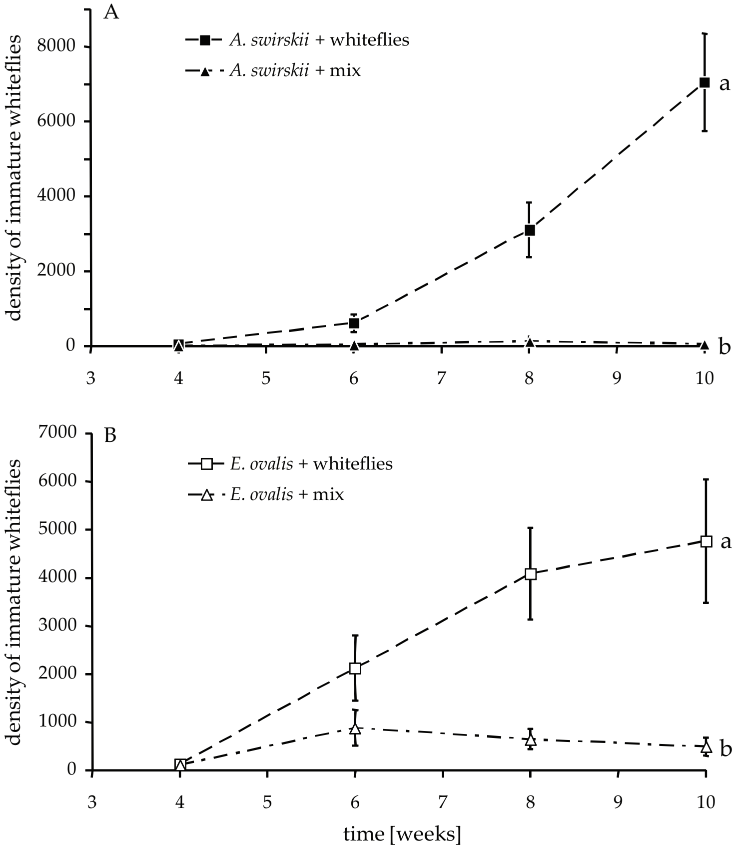

FIGURE 3.2 – The dynamics of greenhouse whitefly on cucumber plants during a 10-week greenhouse experiment in presence of the predatory mites *A. swirskii* (A) and *E. ovalis* (B). Shown are average densities (± SE) of immature whiteflies in the presence (triangles) or absence (squares) of thrips.

Oviposition, juvenile survival and development and predation

The average oviposition rates of *A. swirskii* on a diet of whitefly eggs, first instar thrips or on a mixed diet were not significantly different (FIGURE 3.4; mixed effects model). Predation, however, differed significantly with diet (FIGURE 3.5; thrips: $F_{1,69} = 23.5$, $p<0.0001$, whitefly: $F_{1,66} = 61.6$, $p<0.0001$). When offered the mixed diet, *A. swirskii* consumed roughly half the numbers of each prey, as compared to experiments with single-species diets. All individuals under test consumed the two prey species. Hence, the population of predators tested did not consist of a mixture of individuals that specialized on one prey.

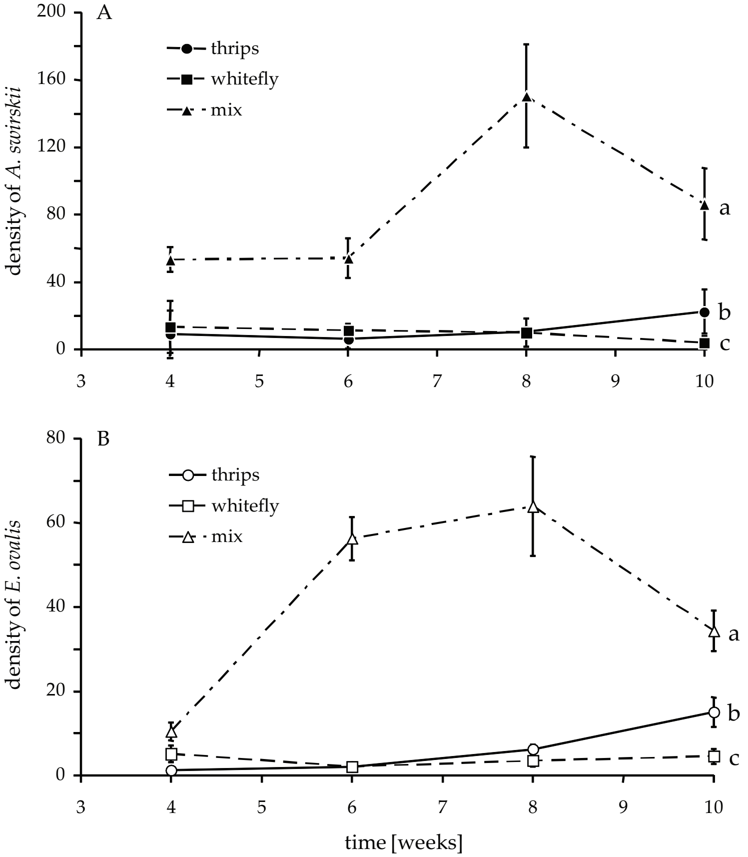

FIGURE 3.3 – The dynamics of predatory mites on cucumber plants during a 10-week greenhouse experiment. Shown are average densities (± SE) of (A) *A. swirskii* and (B) *E. ovalis* with thrips (circles), whitefly (squares) or a combination of thrips and whiteflies (triangles).

Juvenile survival was significantly affected by diet (χ^2 = 10.7, d.f. = 2, p = 0.0047). There was no mortality on a diet consisting of thrips or on the mixed diet, whereas some 34% of the juvenile predators that were feeding on whitefly eggs died. The rate of juvenile development (egg-to-adult) was strongly affected by diet (FIGURE 3.6; log-rank test: χ^2 = 28.9, d.f. = 2, p<0.001). The difference in development was significant among all three diets (FIGURE 3.6; all p's <0.035).

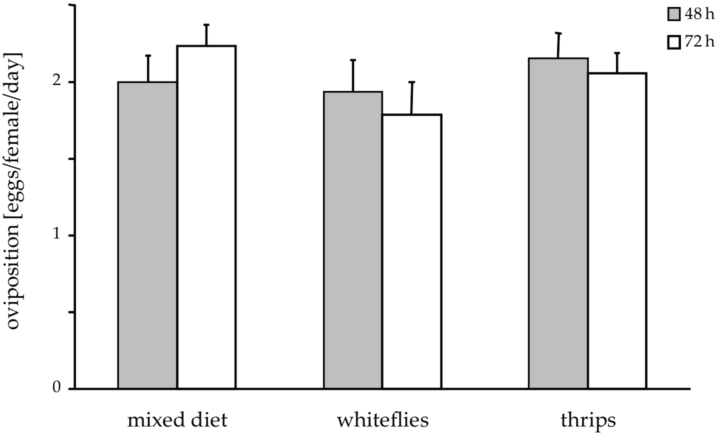

FIGURE 3.4 – Average oviposition rates of young adult females of *A. swirskii* on a diet of thrips larvae, whitefly eggs, or a combination of the two (mixed diet). Shown are average numbers of eggs (+ SE) per female per day measured after 48 and 72 h since the predators were allowed to feed on these prey.

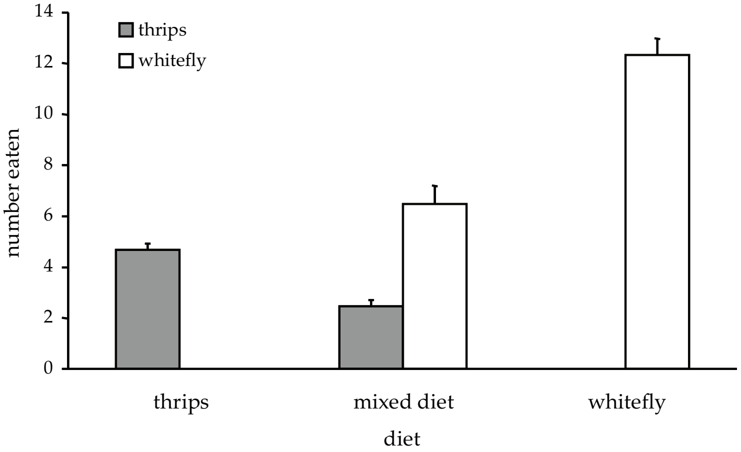

FIGURE 3.5 – Average predation rates by young adult females of *A. swirskii* on thrips larvae and whitefly eggs when offered either of these two prey species separately or in combination (mixed diet). Data are from the same experiment as shown in FIGURE 4. Shown are average number of prey consumed (+ SE) per female per day measured after 48 h since the predators were allowed to feed on these prey.

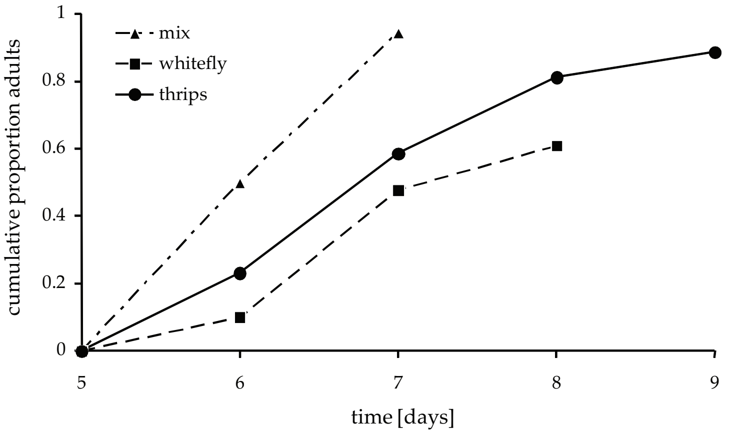

Figure 3.6 – Development of juveniles of the predatory mite *A. swirskii* on a diet of thrips (circles), whitefly (squares) or a combination of thrips and whiteflies (mixed diet, triangles). Shown are the cumulative proportions of juveniles that developed into adults.

Discussion

We investigated whether the use of a single species of natural enemy for biological control of two pests resulted in better control of the two pest species through an increase in predator densities (apparent competition). Alternatively, it could result in temporal escape of one of the two pest species (apparent mutualism). Our results support the first hypothesis and not the second; we found significantly higher abundance of the shared predators and lower levels of whiteflies, one of the two herbivore species. Thus, control of whiteflies was improved by the presence of thrips, but the control of thrips was not affected by the presence of whiteflies. Such asymmetric effects of prey species on each other through a shared predator have also been referred to as indirect amensalism rather than apparent competition (Chaneton & Bonsall, 2000). Perhaps the lack of an effect of the presence of whiteflies on the population densities of thrips was caused by the low initial densities of thrips. Possibly, experiments with higher initial densities of thrips would reveal a positive effect of the presence of whiteflies on the control of thrips.

We found no evidence that either of the two pest species had a positive effect on the other species in the greenhouse. In the laboratory, however, predation rates on each prey was halved in the presence of the other species. This was probably caused by satiation of the predators owing to the higher total density (whitefly plus thrips) of prey. Because the total density of prey in the greenhouse experiments was also higher in the treatments with mixed prey, there might have been similar short-term positive effects of the two prey on each other through satiation of the predators, but they were probably not detected because they were masked by an increase in predator populations during the first four weeks. Experiments should therefore be done in which the populations of prey and predators are sampled more frequently early in the experiment.

Densities of predator species reached much higher levels in presence of both pest species; predator densities were up to 15× higher compared to the situation with only thrips or whiteflies. This may have been partly caused by differences in the amount of food present: in the treatment with *A. swirskii* and thrips, densities of thrips were low (<1 larva/leaf). Hence, the low numbers of predators may have been a direct consequence of low numbers of prey. The density of thrips larvae in combination with *E. ovalis* was always high (>17 larvae/leaf). Thus, the low numbers of this predator cannot have been caused by low prey densities. Moreover, in the treatments with whiteflies and predators, densities of whitefly immatures were always high (>67 immatures/leaf with *A. swirskii* and >138 immatures/leaf with *E. ovalis*), suggesting that food was also not limiting in these treatments. Thus, the high numbers of predators in the treatments with both pests must have another cause besides prey densities.

To assess whether the higher predator densities were caused by differences between a mixed diet and the two single-species diets, we measured oviposition and survival in the laboratory. Oviposition on a mixed diet was not higher than on a diet of each of the two pest species alone. However, pest densities in the laboratory were never limiting for oviposition – the predators consumed at most half of the prey present – whereas densities in the greenhouse might have been. Hence, it cannot be ruled out that higher predator densities in the greenhouse were caused by increased availability of prey. In the laboratory, juvenile survival was affected by diet, and was significantly higher on a mixed diet than on either prey species separately. Likewise, developmental rate was also highest on a mixed diet. These differences in survival and developmental rate were not caused by increased availability of prey in the treatments with the mixed diet, because densities were always sufficiently high to avoid prey shortage. Hence, the higher juvenile survival and developmental rate were a result of the mixture of prey. This better juvenile performance on a mixed diet may also explain the differences in predator density observed in the greenhouse. Assuming exponential population growth of the predatory mites, the small changes in growth rate due to an increase in juvenile survival and developmental rate as observed here, would have enormous effects on numbers of predators: after 8 weeks, the predator population on the mixed diet would be 7× as high as on a single thrips diet and 27× as high as on a single whitefly diet. We suspect that the high densities of *E. ovalis* in the mixed species treatment has a similar cause. However, it must be noted that both thrips and whiteflies were present in sufficiently high numbers in the laboratory experiment, whereas thrips levels in the greenhouse were relatively low compared to whitefly densities. Possibly, a few thrips larvae in a diet of whiteflies already have a strong impact on population growth of the predatory mites. However, it is also possible that most first-instar thrips larvae were consumed by the predators, present in high numbers, shortly after emerging from the eggs, resulting in an underestimate of the density of thrips larvae in the greenhouse.

When comparing *A. swirskii* with *E. ovalis*, it can be concluded that *A. swirskii* is a much better predator of thrips, confirming earlier experiments on cucumber (Messelink et al., 2006), and a better predator of whiteflies when thrips is also present as prey (this experiment). These differences might even be higher in reality because of the free migration of pests between the two predator treatments.

In conclusion, our results demonstrate that increased control of pests can be achieved through apparent competition between two pest species. Whereas earlier studies focused on the use of a non-pest species to increase predator densities (Karban et al., 1994; Hanna et al., 1997; Liu et al., 2006), this study shows that similar results can be reached with two pest species that share a predator species. A further novelty of this study is the indication that the consumption of a mixed diet increases the densities of the natural enemies, not just because of a higher availability of food, but because a mixed diet results in higher growth rates of the predator populations through an increase in juvenile survival, resulting in further reduction of pest densities. We therefore suggest that the effects of mixed diets should be addressed in experimental and theoretical studies of apparent competition.

Nowadays, *A. swirskii* is increasingly used for biological control of thrips and whiteflies in many crops. Biological control strategies in these crops might be improved by tolerating acceptable levels of both thrips and whiteflies in order to stimulate population growth of predatory mites. Further research is needed to clarify relationships between pest levels and yield to enable implementation of results like these.

Acknowledgements

This study was supported by the Dutch Product Board for Horticulture and the Ministry of Agriculture, Nature and Food Quality. RvM received a scholarship of the Technology Foundation (STW project 7180). Maus Sabelis is acknowlegded for many useful comments and stimulating discussions.

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Positive and negative indirect interactions between prey sharing a predator population

G.J. Messelink, R. van Holstein-Saj, M.W. Sabelis & A. Janssen

Prey species can interact indirectly via a shared predator population and, depending on the time scale and the type of dynamics, these interactions can either be negative or positive. For biological control of two pest species by a shared predator population, it is important to consider these indirect pest interactions, which can either disrupt or enhance biological control. We studied the dynamics of two major pest species on greenhouse cucumber: Western flower thrips, *Frankliniella occidentalis* (Pergande) and greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), both attacked by the predator species *Amblyseius swirskii* Athias-Henriot. We investigated how the presence of whiteflies affect the biological control of thrips. Theory predicts that strong population fluctuations can result in long-term positive effects between prey. In a first greenhouse experiment, strong population cycling was induced by releasing high densities of the two pest species at once. In a second experiment this cycling was prevented by releasing lower numbers of whiteflies during several weeks, resulting in a continuous presence of whitefly eggs as food for the predatory mites. Populations fluctuations indeed resulted in long-term positive interactions; a strong increase of the second whitefly generation significantly delayed the suppression of thrips. The reverse was found in the second experiment: repeated releases of whiteflies had a negative effect on thrips populations through a strong numerical response of the predators. Hence, this study proves the potential for both positive and negative predator-mediated interactions between prey, which calls for caution to the biological control of more than one pest species by generalist predators. The results of this study may help us to predict when and how alternative prey affect the dynamics between pests and natural enemies.

Submitted for publication

Generalist predators can cause indirect interactions among prey species that might otherwise not interact (Holt & Lawton, 1994; Janssen et al., 1998; Harmon & Andow, 2004; van Veen et al., 2006; Evans, 2008). If, for example, the density of one prey species increases, the density of the shared predator also increases and consequently, the second prey species might decrease in abundance. Holt (1977) has called this interaction between prey apparent competition, because the dynamics of the two species resemble that of species competing for resources, whereas in fact it is the shared predator that mediates this interaction. Apparent competition is usually defined as a reciprocal negative prey interaction, but most empirical studies show non-reciprocal indirect interactions between prey species (Chaneton & Bonsall, 2000). This means that only one of the two prey species is negatively affected by the predator-mediated prey interaction. Originally, the theory of apparent competition considered equilibrium densities. However, generalist predators can also cause 'short-term' apparent competition between prey species when predators aggregate in habitat patches containing both prey, or when their feeding rate on one prey is enhanced by the presence of another prey (Holt & Kotler, 1987).

The idea that herbivore species may affect each other through shared natural enemies is in particular interesting for enhancing biological control (Janssen et al., 1998; Harmon & Andow, 2004; van Veen et al., 2006). Several studies have indeed shown that the control of a specific pest species can be enhanced by the presence of a second prey through a numerical response of a shared natural enemy (Collyer, 1964; Karban et al., 1994; Bonsall & Hassell, 1997; Hanna et al., 1997; Liu et al., 2006; Messelink et al., 2008, 2010). However, theory predicts that the opposite of apparent competition, apparent mutualism, may also occur (Holt, 1977; Abrams & Matsuda, 1996). This positive indirect effect of one prey population on the other may be detrimental for biological control. These interactions usually occur in the short-term, within a single generation, through satiation or switching behaviour of the shared natural enemy (Murdoch, 1969; Abrams & Matsuda, 1996). Many studies have shown reduced predation rates on a target pest by the presence of alternative prey (short-term apparent mutualism) (Koss & Snyder, 2004; Madsen et al., 2004; Symondson et al., 2006; Xu et al., 2006; Desneux & O'Neil, 2008). In theory, both short- and long-term effects of shared natural enemies can lead to apparent mutualism. Long-term apparent mutualism may occur when population densities of one prey show cycles, resulting in repeated satiation of the shared predators and repeated reduced predation on the other prey (Abrams et al., 1998; Brassil, 2006). However, empirical evidence for this is limited (Tack et al., 2011).

In this study we investigate whether apparent mutualism occurs in an experimental system with two major pest species in greenhouse crops: Western flower thrips, *Frankliniella occidentalis* (Pergande), greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) and their shared predator *Amblyseius swirskii* Athias-Henriot. This predatory mite has proven to be an effective control agent for thrips (Messelink et al., 2006; Arthurs et al., 2009) and whiteflies (Nomikou et al., 2001, 2002; Calvo et al., 2009). We previously showed that apparent competition between thrips and whiteflies is mediated by their shared predator *A. swirskii* on cucumber plants, but positive indirect effects between the pests were not observed (Messelink et al., 2008). Maybe the short generation time of predatory mites (approximately 10 days at 25°C) and the strong numerical response make it hard to observe such effects. One aspect that could strengthen effects of apparent mutualism is the invulnerability of some pest stages for predation, which may induce cycling dynamics. For both thrips and whiteflies it is known that the larger juvenile stages and adults are invulnerable for predation by the predatory mites (Bakker & Sabelis, 1989; Nomikou et al., 2004; Wimmer et al., 2008). Hence, young stages that escape from predation due to predator satiation can easily reach adulthood and create a new generation of offspring, which in turn increases risks on repeated predator satiation and releasing thrips and whiteflies from control. The continuous presence of vulnerable pest stages may counteract population cycling and prevent long-term apparent mutualism. To test this hypothesis, two experiments were designed to see how population dynamics of whiteflies affect the biological control of thrips. In a first greenhouse experiment, strong population cycling was induced by releasing the two pest species at once in high densities. In a second experiment, we tried to prevent this cycling by releasing whiteflies during several weeks, resulting in a continuous presence of whitefly eggs as food for the predatory mites. The results of this study may help us to predict when and how alternative prey affect the dynamics between pests and natural enemies.

Materials and Methods

Cultures

The predatory mite *A. swirskii* was reared in the laboratory on a diet of cattail pollen (*Typha latifolia* L.) on plastic arenas of a type described by Overmeer (1985) (experiment with repeated release), or on *Acarus farris* (Oudemans) and wheat bran (experiment with one release), a method described by Ramakers & van Lieburg (1982). Both cultures were kept in a climate room, under 16 h of artificial illumination per day, at 22°C and 70% RH. Western flower thrips were reared on flowering chrysanthemum plants (*Dendranthema grandiflora* Tzvelev, cv. Miramar) and the greenhouse whitefly was reared on cucumber plants (*Cucumis sativus* L., cv. Shakira, powdery mildew resistant) in separate small greenhouse compartments. Cucumber plants (cv. Shakira) for both greenhouse experiments were grown in rockwool blocks. They were sprayed once with the pesticide abamectine (Vertimec®, Syngenta).

Releases at once

The first greenhouse experiment was conducted to investigate whether strong cycling dynamics of whiteflies can result in longer-term apparent mutualism between thrips and whiteflies in presence of their shared predator *A. swirskii* on cucumber plants. The experiment was carried out in six insect-proof greenhouse compartments of 24 m^2 each. Three compartments were treated with thrips plus predators and three compartments with thrips and whiteflies plus predators. Each compartment contained four rows of plastic sleeved rockwool slabs on which 16 cucumber plants of the sixth-leaf stage were planted. The roots of the plants were preventively treated against *Pythium* spp. with propamocarb (Previcur N®, Bayer Crop Science). Plants were supplied with water and nutrients with drip irrigation and were allowed to grow to a 2 m high wire to support the crop. Side-shoots were removed until the top of the plant reached the crop supporting wire. From then on, two side shoots were maintained per plant and the main shoot was pruned. Cucumber fruits were harvested as soon as they reached the standard size. The total production of cucumbers and cucumbers with severe thrips damage was recorded per compartment during the whole experiment. Thrips damage was considered severe when the fruit was deformed as a result of thrips feeding or showed severe feeding punctures. Slight silver damage was not scored as this is less dramatic for fruit quality. The pest species were released in the first week, shortly after planting. We tried to induce strong cycling by releasing both pest species at once at high densities. Female thrips and whitefly adults (47% females) were collected from the cultures with an aspirator and introduced at densities of respectively 20 and 100 per plant. One week after these pest introductions, female predatory mites were collected in the laboratory with a fine paintbrush and placed on leaf discs of sweet pepper (*Capsicum annuum* L.) (2 cm diameter) containing cattail pollen (*Typha latifolia* L.). One leaf disc with 15 mites was introduced onto each cucumber plant on the 7th leaf from below. Predator and pest densities were monitored during the eight following weeks through weekly collection of six leaves per compartment. Leaves were picked alternating from one of the shoots of two neighbouring cucumber plants that represented a replicate within a compartment. From these plant shoots, we always collected the 8th leaf from the tip. Each leaf was put in a separate plastic bag and transported to the laboratory, where it was cut into strips of about 5 cm wide. The predatory mites and pests were counted on both sides of the leaves using a stereo microscope (40×). Predatory mites were regularly slide-mounted for species identification with the aid of a phase contrast microscope (400×). Only the larval stages of thrips were counted, whereas eggs and larvae of whiteflies were counted separately. The experiment started in April and lasted for 10 weeks. The average temperature and relative humidity during the experiment was 22.9°C and 69%, respectively.

Repeated releases

The second greenhouse experiment was conducted to investigate whether long-term apparent mutualism between thrips and whiteflies can be prevented by counteracting cyclic dynamics through repeated whitefly releases. The experiment was carried out in six of the same greenhouse compartments as described above. Again, we planted 16 cucumber plants in each compartment and these were grown and fruits were harvested and assessed as described above. Three compartments were treated with thrips plus predators and three compartments with thrips and whiteflies plus predators. Thrips adults were released in densities of 20 per plant in the first week as above, but this time, whiteflies were released weekly at densities of 20 adults per plant for a period of 5 weeks, starting in the first week (on average 65% females). Predatory mites were again introduced in the second week as above, at densities op 15 female predatory mites per plant. Pest and predator population densities were assessed as above. The experiment started in February and lasted for 11 weeks. The average temperature and relative humidity during the experiment was 21.3°C and 73% respectively.

Statistical analysis

Differences in population dynamics of thrips and predatory mites among the treatments were analysed in both experiments using linear mixed effects models with time and compartment as random factors to correct for repeated measures and pseudoreplication within compartments. Pest and predator densities were log(x+1) transformed prior to these analyses. Fruit yield and fruit damage was also analysed with linear mixed effects models with the log-transformed total number of fruits and arcsine-transformed fractions of fruit with thrips damage. All statistical analyses were performed using the statistical package GenStat Release 13.2 (Payne et al., 2010).

Results

Releases at once

Thrips densities strongly increased and subsequently decreased in both treatments, but the overall thrips densities were significantly higher in the treatment with whiteflies ($F_{1,4.3} = 21.83$, $p = 0.008$) (FIGURE 4.1A). The delay in suppression of thrips in the treatment with whiteflies compared to the treatment without whiteflies during week number 5-6 corresponded with an increase of immature whiteflies in the whitefly treatment (FIGURE 4.1B). This indicates that apparent mutualism was induced by the reproduction of the second whitefly generation. The addition of whiteflies significantly increased the predator densities compared to the treatment where thrips were the only prey species present ($F_{1,4.7} = 22.2$, $p = 0.006$). The total fruit production did not differ between the treatments ($F_{1,4} = 0.95$, $p = 0.39$), neither did the fractions of fruit damaged by thrips ($F_{1,4} = 0.4$, $p = 0.56$) (TABLE 4.1).

Repeated releases

Differences in thrips densities between the two treatments seem to depend on the time period, with a clear turning point between week number 5 and 6 (FIGURE 4.2A). Thrips densities in the first 5 weeks were higher in the mixed pest treatment than in the treatment with only thrips, but the differences were not significant ($F_{1,4}$ = 2.59, p = 0.18). In the following weeks, thrips densities were significantly lower in the mixed pest treatment than in the treatment with only thrips ($F_{1,4}$ = 24.79, p = 0.008). Hence,

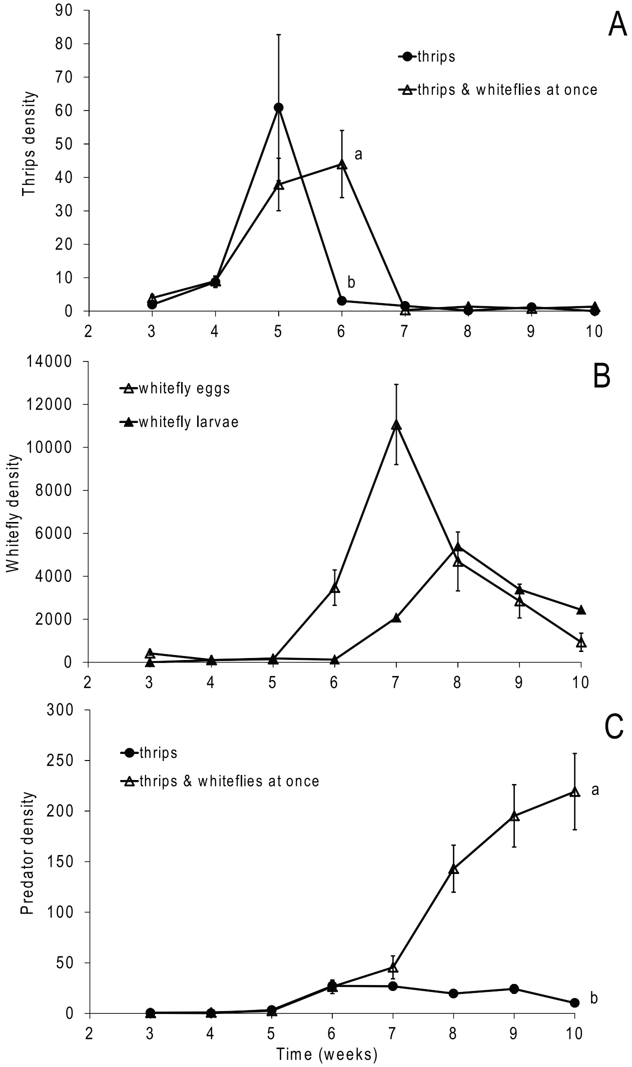

FIGURE 4.1 – Population dynamics of thrips (A), whiteflies (B) and the predatory mite *A. swirskii* (C) on cucumber plants in greenhouses with only thrips or thrips plus whiteflies. The pest species were released in the first week and the predatory mites in the second week. Shown are average densities (± SE) of thrips larvae, whitefly eggs, whitefly larvae and predatory mites (mobile stages) per leaf. Different letters indicate significant differences between treatments across time (p<0.05).

this shows a trend of positive prey-prey interaction in the first 5 weeks (apparent mutualism) and a negative prey-prey interaction in the following weeks (apparent competition). The released whiteflies established well and reached rather constant

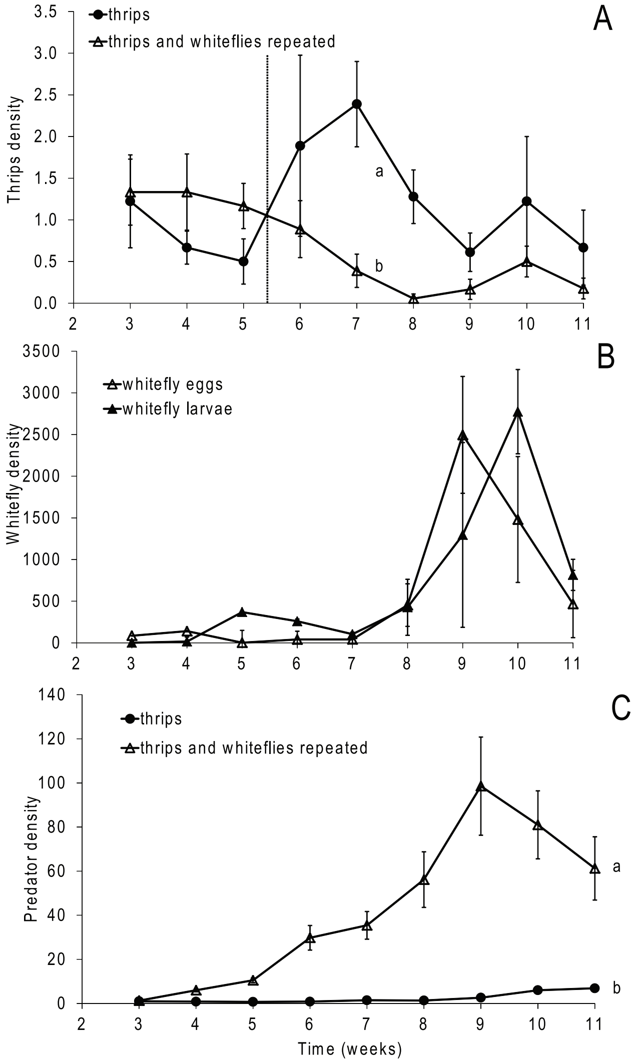

FIGURE 4.2 – Population dynamics of thrips (A), whiteflies (B) and the predatory mite *A. swirskii* (C) on cucumber plants in greenhouses with only thrips or thrips plus whiteflies. Plants were infested with thrips in the first week and with whiteflies weekly during the first 5 weeks. Shown are average densities (± SE) of thrips larvae, whitefly eggs, whitefly larvae and predatory mites (mobile stages) per leaf. Different letters indicate significant differences between treatments across time ($p<0.05$).

TABLE 4.1 – Mean (± SE; n = 16 plants) fruit production and fruit damage by thrips (curved or punctured fruit) during two greenhouse experiments lasting for 10 and 11 weeks. Fruit production did not differ significantly among treatments within each experiment.

Experiment	Treatment	Number of fruits (16 plants)	Damaged by thrips (%)
Releases at once	Thrips	365 ± 16.3	26.2 ± 2.7
	Thrips + whiteflies	347 ± 16.7	26.0 ± 2.4
Repeated releases	Thrips	326 ± 19.7	1.10 ± 0.21
	Thrips + whiteflies	347 ± 10.1	1.25 ± 0.10

population levels from week 3-7, but the population clearly peaked at the end of the experiment (FIGURE 4.1B). The addition of whiteflies to thrips significantly increased the predator densities (FIGURE 4.1C; $F_{1,4}$ = 127.1, p<0.001). We did not find significant differences in fruit production ($F_{1,4}$ = 0.95, p = 0.39) or the fraction of damaged fruits ($F_{1,4}$ = 0.4, p = 0.56) between the two treatments (TABLE 4.1). Thus, although whiteflies did affect thrips densities, this did not affect the overall fruit production and fruit damage.

Discussion

The results of our study support the theory that both positive and negative interactions can occur between prey species that share a natural enemy population. So far, many studies demonstrated effects of apparent competition, but studies showing longer-term apparent mutualism are scarce (Chaneton & Bonsall, 2000). This study is unique in showing that both effects can occur in one experimental system. Short-term apparent mutualism, which occurs within a generation, has typically been shown in studies for predators with a relatively long generation time, such as carabid beetles (Koss & Snyder, 2004; Symondson et al., 2006). In our study system, both the predators and prey species went through several generations. The predatory mite used here has a short generation time: approximately 7 days from egg to adult on a mixed diet of thrips and whiteflies at 25°C (Messelink et al., 2008). Hence, the predators could probably produce 7-8 generations during the experiments. Thrips can produce one generation on cucumber leaves in 14 days at 25°C (van Rijn et al., 1995). Whiteflies have a relatively long generation time of 23 days at 24°C (van Merendonk & van Lenteren, 1978). Based on the average greenhouse temperatures in our experiments, we can assume that thrips went through at least three generations and whiteflies through two generations during the experiments. Thus, the delayed thrips control in presence of whiteflies in the single release experiment can indeed be labelled as long-term apparent mutualism, as these dynamics coincided with a strong increase of the second whitefly generation. These results confirm the theory that strong population fluctuations can result in long-term apparent mutualism

(Abrams et al., 1998). The positive effect of whiteflies on thrips observed here was probably not caused by the predatory mites switching to the more abundant whiteflies. The predatory mites have no preference for either of the two prey species (Messelink et al., 2008), and the two prey have overlapping distributions on the plant, hence, predators encounter them simultaneously. The main reason of the observed delay in thrips suppression is probably mainly caused by the sudden surplus of food by the large second generation of whiteflies, which satiated the predator population. Although the densities of thrips and whiteflies in our experiments did show fluctuations, the dynamics of thrips, whiteflies and predators is stabilized by the presence of invulnerable prey stages (Murdoch et al., 1987; van Rijn et al., 2002). Indeed, when such invulnerable stages are added to the model studied by Abrams et al. (1998), fluctuations dampen out rapidly, and only apparent competition is observed (results not shown). We suspect that the positive effects between prey populations observed here is caused by a combination of predator satiation, invulnerable prey stages, and developmental time lags in the prey and predator populations, which in itself causes cycles in population dynamics (Abrams et al., 1998). In this way, adding high densities of whiteflies resulted in satiation of the predator population, resulting in thrips and whiteflies escaping from predation, which subsequently resulted in an increased next generation of thrips and whiteflies later-on, which again resulted in predator satiation. Preliminary work with a parameter-rich simulation model of thrips and predators (van Rijn et al., 2002) confirms this, but this topic clearly deserves further study.

We hypothesized that strong population fluctuations might be prevented by repeated whitefly releases. This could facilitate the predatory mites through a prolonged supply of whitefly eggs, which are the most suitable prey stages for the predatory mites (Nomikou et al., 2004). The greenhouse experiment with repeated releases of whiteflies indeed showed a negative effect of the presence of whiteflies on thrips populations, and this negative prey interaction can be labelled as apparent competition. The repeated releases of whiteflies resulted in a rapid population increase of the predators, which was apparently high enough to suppress the second whitefly generations and prevent long-term apparent mutualism. A striking difference between the two experiments is the difference in pest and predator densities. The higher average temperatures and light intensity in the single release experiment clearly favoured population increases.

An aspect of apparent competition that has been ignored so far is the effect of a mixed diet on predator populations. Different prey can have complementary nutritional values (Wallin et al., 1992; Toft, 1995; Toft & Wise, 1999; Harwood et al., 2009), thus, the presence of several prey species can increase predator populations due to the higher quality of a mixed diet as well as due to the increased availability of food. This effect of a mixed diet was recently shown for our system: juvenile *A. swirskii* developed significantly faster on a diet of thrips and whiteflies than on either prey species separately (Messelink et al., 2008). The same study showed that juvenile survival and development of *A. swirskii* on thrips alone was significantly higher than on a diet of whiteflies alone. The results of this study confirm these findings. Population densities of *A. swirskii* were much higher with both pest species present than when only thrips were present; up to 21× higher in the single-release experiment and up to 38× higher in the repeated-release experiment.

We studied and discussed predator-mediated interactions between prey, but obviously, our experiments also might include other interactions that we did not observe. Pest species might for example also interact indirectly via the plant they share. Especially whiteflies are known to induce plant resistance (Inbar & Gerling, 2008). Such interactions might have played a role in our experiments as well, but this was beyond the scope of our study. Also resource competition between thrips and whiteflies cannot be excluded because we did not include control treatments without predators. However, such competition is not likely because we did our experiments on full-grown plants which had relatively large leaf surfaces, hence, there was enough food for both prey species.

The results of this study are important for crop protection. They confirm our earlier studies, where we show that the presence of more than one pest species can enhance pest control by the numerical response of the predatory mites (Messelink et al., 2008, 2010). However, this study shows the risks of pests escaping temporary from control by satiation effects of the predator population. Such effects might, for example, occur when pests species migrate from old plants to young plants when a new cropping cycle is started. As only the adults migrate (flying), this may induce strong cycling and generate long-term apparent mutualism, as shown in this study. The risks of such whitefly population peaks and subsequent effects on predator satiation can possibly be prevented by releases of natural enemies that attack older whitefly stages that are less vulnerable for predation by *A. swirskii*, such as the parasitoid *Encarsia formosa* (Gahan). Although this study demonstrated significant differences in thrips control, this did not result in significant differences in fruit damage. Other studies showed a clear linear relationship between thrips densities and fruit damage (Shipp et al., 2000), but the differences in thrips densities in this study were possibly not high enough to give such effects.

In summary, we have demonstrated that indirect interactions between prey sharing a predator population can either be negative or positive, depending on the time-scale and type of dynamics. Predatory mites showed to be excellent model organisms for detecting such interactions within the time-scale of one cropping cycle, because of their short generation time and strong numerical response. Furthermore, we provide additional evidence for the positive effects of mixtures of prey for predator development (see also Messelink et al., 2008). Hence, we suggest that the numerical response of predators to prey mixtures deserves more attention when studying indirect prey interactions within ecosystems, e.g., for further developing pest control strategies in agro-ecosystems.

Acknowledgements
This study was supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation. E. de Groot, W. van Wensveen, and L. Kok are thanked for their assistance in the greenhouse experiments.

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Pest species diversity enhances control of spider mites and whiteflies by a generalist phytoseiid predator

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To test the hypothesis that pest species diversity enhances biological pest control with generalist predators, we studied the dynamics of three major pest species on greenhouse cucumber: Western flower thrips, *Frankliniella occidentalis* (Pergande), greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), and two-spotted spider mites, *Tetranychus urticae* Koch in combination with the predator species *Amblyseius swirskii* Athias-Henriot. When spider mites infested plants prior to predator release, predatory mites were not capable of controlling spider mite populations in the absence of other pest species. A laboratory experiment showed that predators were hindered by the webbing of spider mites. In a greenhouse experiment, spider mite leaf damage was lower in the presence of thrips and predators than in the presence of whiteflies and predators, but damage was lowest in the presence of thrips, whiteflies and predators. Whitefly control was also improved in the presence of thrips. The lower levels of spider mite leaf damage probably resulted from (1) a strong numerical response of the predator (up to 50× higher densities) when a second and third pest species were present in addition to spider mites, and (2) from *A. swirskii* attacking mobile spider mite stages outside or near the edges of the spider mite webbing. Interactions of spider mites with thrips and whiteflies might also result in suppression of spider mites. However, when predators were released prior to spider-mite infestations in the absence of other pest species, but with pollen as food for the predators, we found increased suppression of spider mites with increased numbers of predators released, confirming the role of predators in spider mite control. Thus, our study provides evidence that diversity of pest species can enhance biological control through increased predator densities.

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Much research has been devoted as to whether the presence of multiple natural enemies leads to more efficient pest suppression than the presence of single enemy species (Rosenheim et al., 1995; Denoth et al., 2002; Cardinale et al., 2003; Casula et al., 2006). Empirical studies show that increasing diversity of natural enemies can result in a full spectrum of outcomes, including additive, antagonistic, synergistic, or no effects on biological control (Casula et al., 2006; Janssen et al., 2006, 2007). Less attention has been paid to the impact of pest species diversity on biological control. Most pest management programs in modern greenhouse cropping systems are focused on excluding and eliminating pest species as much as possible, resulting in low pest species diversity. However, indirect interactions occurring among various pest species may enhance biological control (Janssen et al., 1998; Harmon & Andow, 2004; Prasad & Snyder, 2006; van Veen et al., 2006).

Such an indirect interaction occurs when the density of one prey species affects the density of a polyphagous natural enemy, which consequently affects the density of a second prey species. Holt (1977) was the first to develop theory on this mode of indirect interaction. He showed that the equilibrium density of a population of one prey species decreases when that of another, non-competing prey species is increased. Holt coined the term ‘apparent competition’ because it appears as if the two species compete for a shared resource, whereas in fact the two prey populations interact via the shared predator. Subsequently, theory was developed for the case of short-term dynamics of systems involving multiple prey that share the same natural enemy (Holt & Kotler, 1987; Abrams & Matsuda, 1996; Abrams et al., 1998). Such short-term, non-equilibrium dynamics are a more realistic scenario in agricultural systems with a short production cycle than the equilibrium dynamics studied by Holt (1977) and Holt & Lawton (1994). The theory on short-term dynamics shows that predators can not only mediate apparent competition between two of their prey species, but also apparent mutualism. In the latter case, predator satiation results in a short-term positive indirect interaction between its prey species. With respect to biological control, some studies have indeed demonstrated that the control of a pest species can be improved by the presence of another pest species (Collyer, 1964; Karban et al., 1994; Liu et al., 2006; Messelink et al., 2008), whereas disruption of biological control through predator satiation in the short-term has also been demonstrated (Koss & Snyder, 2005; Symondson et al., 2006).

So far, both theory and experiments on the effects of a shared predator have ignored the effects of a mixed diet on predator populations. Different prey can have complementary nutritional values (Wallin et al., 1992; Toft, 1995, Evans et al., 1999), and this can amplify the effects of predator-mediated apparent competition. Hence, the presence of several prey species can increase predator populations through the increased availability of food as well as through the higher quality of a mixed diet. Based on these mechanisms, pest species diversity in combination with predators attacking various prey species can enhance biological control (Messelink et al., 2008).

This study was designed to further evaluate the hypothesis that increasing pest species diversity can enhance biological control with generalist predators. We studied the dynamics of three major pest species in greenhouse crops, i.e., Western flower thrips, *Frankliniella occidentalis* (Pergande), greenhouse whiteflies, *Trialeurodes vaporariorum* (Westwood), two-spotted spider mites, *Tetranychus urticae* Koch and the predator *Amblyseius swirskii* Athias-Henriot (Zannou et al., 2007). The predatory mite

A. swirskii has proven to be an effective control agent for thrips (Messelink et al., 2006) and whiteflies (Nomikou et al., 2001, 2002), whereas its effect on spider mites is still unclear. Moreover, control of whiteflies is improved when thrips are present in low densities (Messelink et al., 2008). Although *A. swirskii* does feed on spider mites (Momen & El-Saway, 1993), greenhouse observations suggest that the webbing produced by spider mites impedes effective control because *A. swirskii* is not able to enter it (Messelink, personal observations). It has been suggested that one of the functions of spider mite webbing is defence against predators, and the way in which predatory mites cope with this webbing is suggested to depend on the dorsal chaetotaxy of the predators (Sabelis & Bakker, 1992). The predator *A. swirskii* has short dorsal setae, and is therefore expected to be hindered by spider mite webbing. We first verified this by measuring predation rates of spider mite eggs by *A. swirskii* in the presence and absence of spider mite webbing. Subsequently, we verified that *A. swirskii* is not capable of controlling spider mite populations in the absence of other pest species. Finally, we investigated whether spider mite control by *A. swirskii* can be enhanced by the presence of the other pest species, i.e., thrips and whiteflies. A further experiment was done to shed some light on the possible mechanisms responsible for increased spider mite control in the presence of other pests. It is not our aim here to completely disentangle how multiple pest species interact, i.e., directly, indirectly via the plant, indirectly via the shared predator or via any combination of these mechanisms. Our primary goal is to establish the extent to which multiple pest species affect control efficacy and discuss the interactions mentioned.

Materials and Methods

Cultures

The predatory mite *A. swirskii* was reared on a diet of cattail pollen (*Typha latifolia* L.) in climate rooms, under 16 h of artificial illumination per day, at 22°C and 70% RH on plastic arenas of a type described by Overmeer (1985). For the experiment with predator densities, *A. swirskii* was obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands). These mites were reared on bran containing the sugar mite *Carpoglyphus lactis* L. All prey/pest species were reared on plants in greenhouse compartments. Two-spotted spider mites were reared on bean plants, *Phaseolus vulgaris* L., Western flower thrips were reared on flowering chrysanthemum plants (*Dendranthema grandiflora* Tzvelev cv. Miramar) and the greenhouse whitefly was reared on tobacco plants (*Nicotiana tabacum* L.). For assessing the effects of spider mite webbing on predatory mites in the laboratory, we reared the predatory mite species *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor) on spider mite-infested cucumber plants in greenhouses.

Effect of webbing on predation

A laboratory experiment was set up to assess the extent to which spider mite webbing hinders predation by *A. swirskii.* Although spider mite eggs are less suitable for development of *A. swirskii* than the mobile stages of this prey (R. van Maanen, personal observations), we used eggs because they do not move from the web where they are deposited. Predation by *A. swirskii* was compared with that of two other species of predatory mite, *P. persimilis* and *N. californicus*, commonly used for control of spider mites. This comparative test served to assess the impact of webbing on predation of spider mite eggs. We placed cucumber leaf discs (3 cm diameter, cut from the inter-vein area of 4-week-old plants) upside down on water-saturated cotton wool in plastic boxes (14 × 20 cm), six leaf discs per box. Three female spider mites were placed on each leaf disc for 2 days, resulting in a colony with 40 eggs on average. Spider mites were prodded with a small paintbrush to make them move out of the webbing without harming the web structure, and the eggs were counted. The webbing was removed from half of the leaf discs with a fine needle. Single, young adult female predators (1-6 days old since their last moult, starved for 1 day), were placed on the leaf discs and the boxes with discs were incubated in a climate room (25°C and 16/8 L/D). The surviving eggs were counted after 24 h. Treatments were replicated 12× with new predator individuals. Differences between treatments involving web or web removal and treatments involving different predator species were analysed using an ANOVA on the log-transformed numbers of eggs, followed by Fisher's LSD (Least Significant Difference) test at the 5% confidence level.

Spider mite control by *Amblyseius swirskii*

We studied the population dynamics of spider mites and *A. swirskii* in the absence of other pest species on cucumber plants (cv. Aviance, powdery mildew resistant) in two separate greenhouse compartments (18 m^2). So there was only one treatment, in which the spider mites were released prior to the predatory mites. Each compartment contained two tables (1 × 3 m) on which plants were grown up to a 1.5 m high wire to support the crop. Plants were grown in rock wool blocks. Side-shoots were removed until the top of the plant reached the crop supporting wire, and all further plant shoots were suspended from the wire.Each greenhouse compartment had a small entrance corridor, and was ventilated with an air pressure system in order to minimize contamination by organisms from outside. The plants had six leaves when the experiment started in March 2006. Roots were preventively treated against *Pythium* spp by soaking the rock wool blocks in a 0.1% solution of propamocarb (Previcur N®, Bayer Crop Science). No further pesticides were used. Four plants were placed on two pieces of rock wool substrate on each table. The rock wool mats were continuously immersed in a nutrient solution that was automatically supplied twice a day. One day after planting, the plants were infested with spider mites by adding two cucumber leaf discs (2 cm diameter), each containing 10 females that were collected from the culture. These leaf discs were put on the fourth and fifth leaf of each plant, counted from the lowest leaf. Predatory mites were released seven days after introducing the spider mites. Female predatory mites were collected in the laboratory with a fine paintbrush and placed on leaf discs of sweet pepper (*Capsicum annuum* L.) (2 cm diameter) containing cattail pollen. One leaf disc with 15 mites was introduced onto each cucumber plant on the 7th leaf from below. Each table was considered as a single replicate. Hence, there were four replicates.

The experiment lasted 11 weeks, roughly corresponding to the standard cropping period for modern glasshouse cultures. Cucumbers were harvested as soon as they reached the standard fruit size, but fruit yield was not measured. The numbers of predatory mites and the percentage of leaf surface with spider mite damage were assessed 5, 7, 9 and 11 weeks after introducing the pest species. Assessment of the populations was done on six leaves of each replicate. The leaves were collected by randomly choosing three shoots of which the sixth and eighth leaf, counted from the tip, were collected. Each leaf was put in a separate plastic bag and transported to the laboratory, where it was cut into strips of 5 cm wide. The predatory mites were counted on both sides of the leaves using a stereomicroscope (40×). Spider mite damage was assessed by estimating the percentage of leaf damage by persons which were trained for these observations with the computer program 'Distrain' (Tomerlin & Howell, 1988).

Effects of prey diversity on pest control

The combined control of whiteflies, thrips and spider mites by *A. swirskii* was studied on plants in greenhouse compartments as explained above and with various combinations of pests: (1) spider mites plus thrips; (2) spider mites plus whiteflies; (3) spider mites plus thrips and whiteflies; (4) thrips only. Combinations of whitefly and thrips were examined in an earlier experiment, described elsewhere (Messelink et al., 2008). Experiments were carried out in eight compartments simultaneous with the experiment on spider mite control. Each treatment was replicated four times.

For all treatments with thrips, female thrips were collected with an aspirator from a culture on chrysanthemum and introduced at a rate of 10 per plant. For all treatments with whiteflies, adult greenhouse whitefly (sex ratio 1:1.27 male: female) were collected with an aspirator from a culture on tobacco plants and released at a rate of 40 per plant. All pest species except spider mites were released one day after planting. Predatory mites (15 females per plant) were released seven days after introducing the pests, at the start of the second week (thus, exactly at the same time and same number as in the experiment on spider mite control). Spider mites were introduced three weeks later than the predatory mites, in order to evaluate the effects of an already established predator population. A period of 3 weeks was chosen to allow the predator populations to increase on the food present in the crop (thrips, whiteflies or both). Labelled cucumber leaves of young side shoots were provided with small cucumber leaf discs (2 cm diameter), each containing 20 female spider mites collected from the culture on bean plants. These discs with spider mite colonies were applied to four leaves per replicate. During the next six weeks, we assessed the percentage of spider mite damage on four younger and four older leaves, next to the leaves on which the spider mites were released. Assessments per leaf were done as described in the experiment on spider mite control by the same trained person, as it was impossible to count mites on so many plants using a non-destructive method. The typical leaf tissue damage caused by spider mites (Park & Lee, 2002) could easily be distinguished from thrips damage. Heavily infested and desiccating/wilting leaves were considered as 100% damaged by spider mites. A control treatment with spider mites added to plants with only predatory mites could not be included, because predatory mites do not survive on plants in a period of three weeks without any prey. The numbers of predatory mites, thrips and whiteflies were assessed 5, 7, 9 and 11 weeks after starting the experiment (and introducing the pest species). Assessment of the populations was done on six leaves in each treatment, as described above. The number of predatory mites, thrips and whiteflies were counted on both sides of the strips of leaf using a stereomicroscope (40×). Predatory mites were regularly slide-mounted for identification to species with the aid of a phase contrast microscope (400×). Only the juvenile stages of thrips and whiteflies were counted because adults fly away when leaves are collected. When the densities of whiteflies exceeded 500 individuals per leaf, densities were assessed only on representative parts of the underside of each leaf, and then extrapolated to the whole surface of the leaf. The average temperature and relative humidity were comparable among greenhouse compartments (22°C and 74% RH).

For statistical analyses, a repeated measures ANOVA was performed on the arcsine square-root transformed fractions of estimated leaf damage by spider mites. The time since introduction of the pest organisms was chosen as the repeated measure variable. The same repeated measures analyses were performed for densities of thrips, whiteflies and predators after a log(x+1) transformation. Differences between treatments were tested at a 5% confidence level using Fisher's LSD method.

Effects of predator density on spider mite control

A third greenhouse experiment was carried out to test the effects of densities of *A. swirskii* on the establishment and population dynamics of spider mites in a situation where the predators were introduced 1 week prior to spider mite infestation. Differences in spider mite densities among treatments in the former experiment might not only be caused by predator densities, but also by interactions of thrips and whiteflies with spider mites, such as resource competition, induced plant resistance or predation by thrips. We therefore released predators in two densities prior to spider-mite infestations in the absence of the other two pest species. In this way, the effects of thrips and whitefly presence on spider mite densities through the shared predator population was mimicked, while excluding the other interactions between spider mites and the other pests. In one greenhouse compartment of 24 m^2, we placed eight cucumber plants (cv. Filia, powdery mildew resistant) on rockwool mats on each of three tables (1.5 × 3 m). These plants were treated once with Abamectine when they were two weeks old, to keep them free of thrips. Plants were grown as in the experiments described above. Each plant was isolated and did not touch other plants and was allowed to grow up to a 2 m high wire that supported the plant. When the plants were 4 weeks old, with 7-8 full-grown leaves, we divided the plants into three groups, and treated them with (1) no predatory mites (control), (2) a low density of 50 predatory mites per plant, and (3) a high density of 500 predatory mites per plant.

The predatory mites were released as a mixture with bran and the sugar mite *C. lactis* (Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands), and they were deposited on top of the rockwool blocks near the base of the plants, from where the mites were able to walk up the plants. The few sugar mites present were not observed to walk onto the plants. We released the predators one week prior to the spider mite infestations to allow them to colonize the plant. Because the plants were devoid of prey, and predatory mites do not survive on plants without food, we added 20 µg of pollen of *T. latifolia* as food for the predators onto all plants on the 7th leaf from below. Plants with the same treatment were placed on one table to avoid contamination among treatments. The dispersal of mites among plants was impeded by placing sticky plates around the rockwool slabs on which the plants were standing. Each plant was considered as one replicate. One week after the predator releases, all plants were infested with spider mites on the 9th leaf (counted from below) by adding one cucumber leaf disc (2 cm diameter), each containing 20 females, collected from a culture on bean plants. The numbers of spider mites and predatory mites were assessed on these leaves 2 weeks later by cutting them and counting the mites using a stereo microscope (40×) as described in the greenhouse experiments above. The average greenhouse temperature was 22°C and the average relative humidity 74%. Effects of treatments on spider mite densities were analysed using an ANOVA on the log(x+1)-transformed numbers of the sum of eggs and mobile stages. Differences between treatments were tested at a 5% confidence level using Fisher's LSD method.

Results

Effect of webbing on predation

Spider mite webbing had a significant effect on the predation of spider mite eggs by *A. swirskii* ($F_{5,64} = 20.68$, $p<0.001$); predation was reduced by 57% (Figure 5.1). The presence of webbing had no impact on predation by the predatory mites *P. persimilis* and *N. californicus*. In the absence of webbing, the predation rate of *A. swirskii* was also lower than that of *P. persimilis* (Figure 5.1). The maximum predation rate of *P. persimilis* might even be higher than observed here, because spider mite eggs were almost depleted in some replicates. In the *N. californicus* and *A. swirskii* treatment, ample amounts of eggs were available throughout the experiment.

Spider mite control by *Amblyseius swirskii*

Amblyseius swirskii was unable to control spider mites on cucumber plants without other pest species (Figure 5.2). At the end of the experiment, the plants were completely covered by spider mite webbing. Crop growth was poor and many leaves were desiccated. Though *A. swirskii* was able to establish, densities remained low (<1.3/leaf), at least until week 9 (Figure 5.2). At the end of the experiment, a mild contamination with thrips was observed in all replicates (average 0.2 and 0.3 larvae/leaf in respectively week 9 and 11), which may explain the increase in predator densities (Figure 5.2).

Effects of prey diversity on pest control

When spider mites were released on plants with thrips, whiteflies or thrips plus whiteflies, there was a strong effect of pest treatment on leaf damage by spider mites

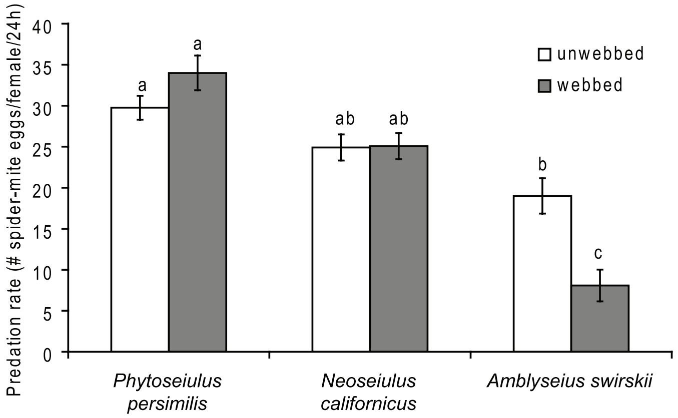

Figure 5.1 – Predation rates of three predatory mite species on two-spotted spider mite eggs on cucumber leaf discs with (grey bars) or without (white bars) spider mite webbing. Shown are average numbers of spider mite eggs consumed (± SE) per female predatory mite in 24 h. Different letters indicate significant differences among treatments (Fisher's LSD test, p<0.05).

(FIGURE 5.3), resulting in significant differences among treatments ($F_{2,42} = 19.97$, $p<0.001$). The highest levels of spider mite damage were observed in the treatment with whiteflies, spider mites and *A. swirskii*. Damage was lower in the treatment with thrips, spider mites and *A. swirskii*, and the lowest levels of spider mite damage were found when both thrips and whiteflies and *A. swirskii* were present (FIGURE 5.3).

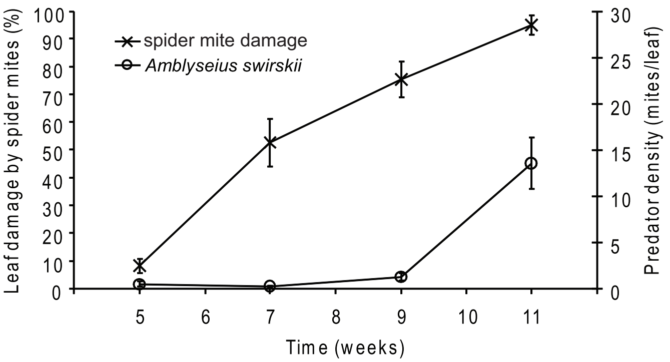

FIGURE 5.2 – The dynamics of two-spotted spider mite damage and of numbers of predatory mites (*A. swirskii*) during an 11 week greenhouse experiment. Spider mites were added at the start of the experiment (week 1), predators were added in the second week. No other pest species were released. Shown are average percentages (± SE) of leaf damage and average densities (± SE) of the predatory mite *A. swirskii*.

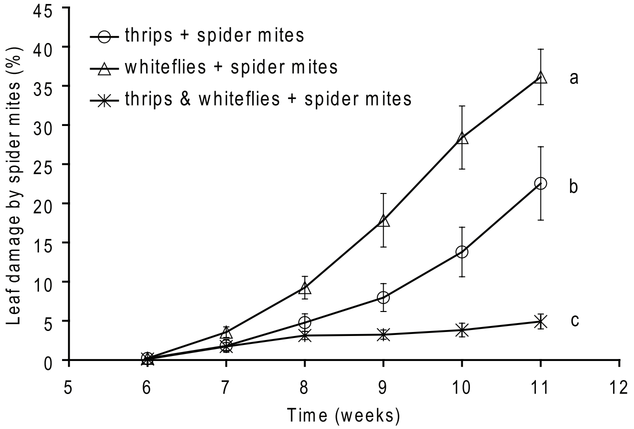

FIGURE 5.3 – Leaf damage by two-spotted spider mites in a greenhouse in the presence of the predatory mite *A. swirskii* and the pest species Western flower thrips, greenhouse whiteflies or Western flower thrips plus greenhouse whiteflies. Shown are average percentages (± SE) of leaf damage on eight marked leaves that neighboured the leaves where spider mites were released. Different letters indicate significant differences among treatments through time (Fisher's LSD test, $p<0.05$).

There was a significant effect of pest species diversity on the densities of predators (FIGURE 5.4) ($F_{3,92}$ = 88.45, $p<0.001$). The highest predator levels were found in the treatments with thrips, whiteflies and spider mites, where predator levels were at least 11× higher than in the other treatments at the population peak in week 9 (FIGURE 5.4). Two replicates of the whitefly treatment were slightly contaminated with thrips at the end of the experiment (on average 0.8 larvae/leaf in week 11), but this was ignored in the statistical analyses.

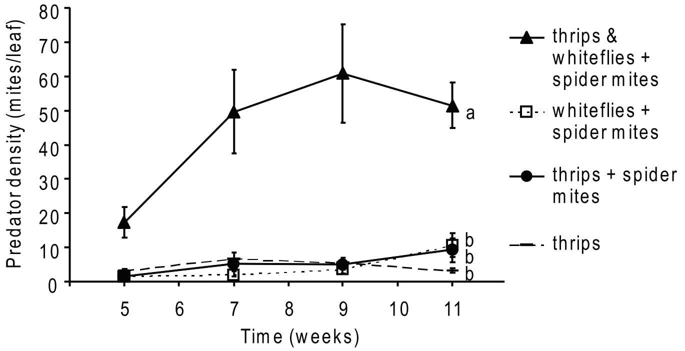

FIGURE 5.4 – The dynamics of predatory mites on cucumber plants during an 11-week greenhouse experiment. Shown are average densities (± SE) of the predatory mite *A. swirskii* on plants with only Western flower thrips, Western flower thrips plus two-spotted spider mites, greenhouse whiteflies plus two-spotted spider mites, and Western flower thrips plus greenhouse whiteflies plus two-spotted spider mites. Different letters indicate significant differences among treatments through time (Fisher's LSD test, p<0.05).

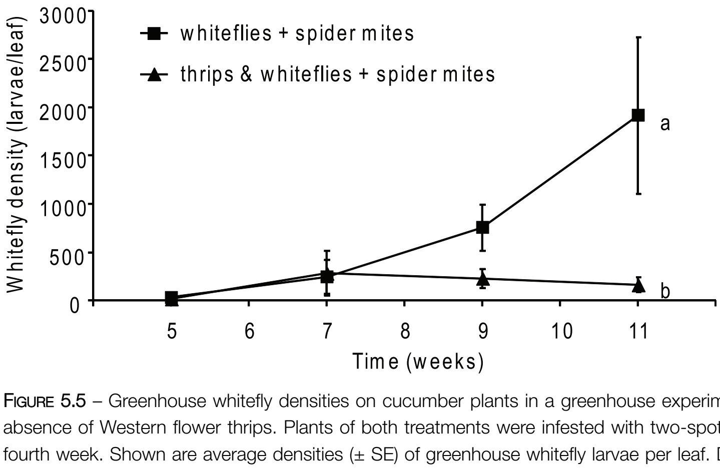

FIGURE 5.5 – Greenhouse whitefly densities on cucumber plants in a greenhouse experiment in the presence or absence of Western flower thrips. Plants of both treatments were infested with two-spotted spider mites in the fourth week. Shown are average densities (± SE) of greenhouse whitefly larvae per leaf. Different letters indicate significant differences among treatments through time (Fisher's LSD test, p<0.05).

Not only spider mites, but also whiteflies were controlled significantly better in the presence of thrips (FIGURE 5.5) ($F_{1,43}$ = 40.77, $p<0.001$), confirming the results of a similar experiment to which no spider mites were added (Messelink et al., 2008). Thrips densities did not differ significantly among treatments ($F_{2,66}$ = 0.01, p = 0.99) and were always controlled adequately (FIGURE 5.6), as in experiments reported elsewhere (Messelink et al., 2008).

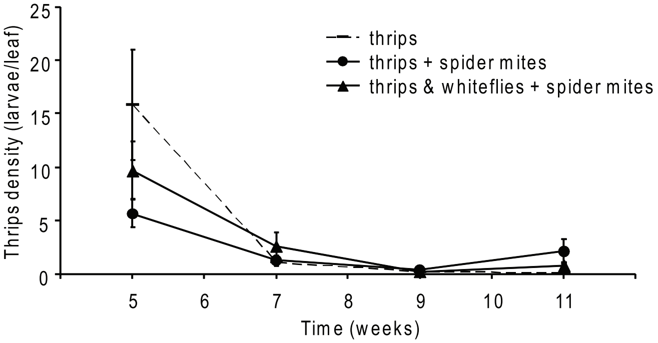

FIGURE 5.6 – Densities of Western flower thrips on cucumber plants in a greenhouse experiment in the presence or absence of greenhouse whiteflies and two-spotted spider mites. Shown are average densities (± SE) of Western flower thrips larvae. Differences among treatments were not statistically significant (Fisher's LSD test, p<0.05).

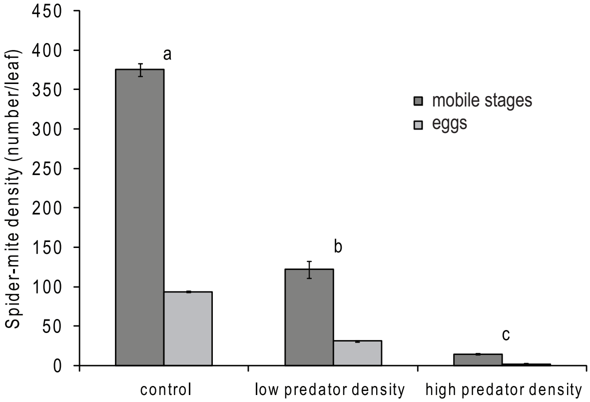

FIGURE 5.7 – Two-spotted spider mite densities on cucumber plants on which no, low or high densities of the predatory mite *A. swirskii* were released prior to spider mite infestation. Shown are average densities (± SE) of mobile stages and eggs of spider mites per leaf, two weeks after the plants were infested with 20 females of spider mites per leaf. Different letters indicate significant differences among treatments for the sum of egg and mobile stages (Fisher's LSD test, p<0.05).

Effects of predator density on spider mite control

The release of predatory mites prior to spider mite infestation significantly affected densities of spider mites (FIGURE 5.7) ($F_{2,20} = 32.77$, $p<0.001$). The establishment of spider mites was even prevented on some plants on which high densities of predatory mites were released. The average predatory mite densities in the treatments with low and high predator releases were 7.3 and 11.1 mites/leaf, respectively.

Discussion

We tested the hypothesis that higher diversity of pest species enhances biological control with generalist predators. Indeed, in the presence of the generalist predatory mite *A. swirskii*, spider mite leaf damage was significantly lower in the presence of both thrips and whiteflies, than when there were either thrips only or whiteflies only. Spider mite leaf damage was reduced more in the presence of predatory mites plus thrips than with predatory mites plus whiteflies. In the absence of other pest species or other alternative food such as pollen, *A. swirskii* was clearly not able to control spider mites and there was hardly any growth of the predator populations.

The exact mechanisms responsible for this strong reduction in spider mite leaf damage in the presence of other pest species cannot be inferred from our experiment; a combination of direct and indirect interactions among the pests can be involved. Direct effects of whiteflies on spider mites are not likely to occur (e.g., reciprocal predation has never been observed), but competition for resources among whiteflies and spider mites might have reduced the growth of spider mite populations. However, because of the high availability of undamaged cucumber leaf tissue, we assume this to be of minor importance. If resource competition did strongly affect the population growth of spider mites, we would expect higher levels of spider mite leaf damage in the treatment with lower whitefly levels. However, the opposite occurred: lower levels of spider mite leaf damage were found at lower levels of whiteflies. This suggests that some other mechanism suppressed spider mites, such as indirect interactions of whiteflies with spider mites via the plant or via the predator. Unlike whiteflies, thrips do not only act as herbivores, but also as predators of spider mite eggs (intraguild predation; Trichilo & Leigh, 1986). These direct effects on spider mites might explain the lower densities of spider mites in the presence of thrips than in the presence of whiteflies. As predator densities did not differ significantly between these two treatments (FIGURE 5.4), it is less likely that predator densities are responsible for the strong difference in effects on spider mites. Competition for food between the spider mites and the thrips is also not likely to have occurred, because thrips levels were quite low (<5 larvae/leaf). However, other studies have shown that thrips hide inside the webbing produced by spider mites when predators are present (Pallini et al., 1998; Venzon et al., 2000), so that local competition between thrips and spider mites might have played a role. Nevertheless, it is likely that the lower levels of spider mite leaf damage were a result of predatory mites as well as thrips feeding on spider mites. The hiding of thrips in the spider mite webbing could also result in reduced control of thrips (Magalhães et al., 2007), but we found no evidence for this, possibly because the amount of spider mite webbing was too low.

Whiteflies and thrips might have reduced population growth rates of spider mites indirectly via the plant, as attacks of plants by one pest species can induce resistance mechanisms in the plant, which can subsequently slow the population growth of a second pest species (Karban & Carey, 1984). This so-called induced resistance has potential for improving biological control (Karban et al., 1997). Further experiments are needed to clarify if such induced resistance occurs among the pest species in this study. Induced resistance might even have affected the consumption of spider mite eggs by thrips. On cotton, it was shown that induced plant resistance caused thrips to shift more towards predation than herbivory (Agrawal et al., 1999), but the reduced density and quality of spider mites on induced plants may antagonize this shift towards increased predation (Agrawal & Klein, 2000).

The second indirect interaction between the pests that possibly resulted in lower levels of spider mite leaf damage is mediated by the shared predator. Our laboratory experiment shows that, despite the predators experiencing hinder from the spider mite webbing, predation of spider mite eggs still occurred. We decided to use spider mite eggs because they cannot escape from the web, but in the greenhouse, all stages of spider mites were present. We assume that the effects of the predators on spider mites in the greenhouse were mainly based on consumption of mobile stages outside or near the edges of spider mite webbing.

Reduction of spider mite leaf damage in the presence of other pests may well arise as a consequence of the strong numerical response of the predator when a second or third pest species was present in addition to the spider mites. Indeed, predator densities were higher when thrips and whiteflies were present together with spider mites, with up to 50× higher densities when both thrips and whiteflies were added. The experiment where two densities of predatory mites were released prior to spider mite infestations clearly shows that higher predator densities reduce spider mite densities more than lower predator densities. So besides all other possible direct and indirect pest interactions, we suggest that predator densities are to a large extent responsible for the improved suppression of spider mites in the presence of other pest species. Not only spider mites, but also whiteflies were better controlled at higher predator densities due to the presence of both thrips and whiteflies. These predator-mediated interactions among the three pest species can be classified as apparent competition, with the addition of thrips to a system of spider mites and whiteflies resulting in lower levels of spider mite leaf damage and better control of whiteflies.

In addition to these density effects, several trait-mediated effects might have occurred as well. These occur when one species modifies the interaction between a pair of species by changing the behaviour of individuals of one or more of these species (not their numbers) (Prasad & Snyder, 2006). We suggest that thrips larvae inside spider mite webbing may have caused spider mites to move out of the webbing, thereby making them more susceptible to predation.

Our results suggest that generalist phytoseiid mites such as *A. swirskii* can play an important role in reducing the colonization of a crop by spider mites, even when they are incapable of controlling spider mites alone. To which extent *A. swirskii* can control starting colonies of spider mites depends, at least partly, on the predator densities at the time of infestation, and thus of the presence of food for sustaining predator populations. The sequence of crop infestation by different pest species is therefore very important for the control of spider mites by *A. swirskii*, at least in crops where alternative food sources such as pollen are not available. Once spider mites have formed colonies, generalist predators such as *A. swirskii* cannot control them, and more specialized spider mite predators, such as *P. persimilis*, will be needed for spider mite control.

The higher predator densities in the treatments with more than one pest species may not have been merely caused by increased prey availability alone. Previous experiments showed that juveniles of *A. swirskii* survive and develop better on a mixed diet of thrips and whiteflies than on a single diet of either of these species. These effects of a mixed diet were suggested to be responsible for strong increases in predator densities in greenhouses in which both thrips and whiteflies were present, and consequently, for lower densities of whiteflies in the presence of thrips (Messelink et al., 2008). The high predator densities observed in the treatment with thrips, whiteflies and spider mites together support this idea. In theory, the addition of spider mites to a menu of thrips or whiteflies could have had the same effect, but the present results show no evidence for this: the addition of spider mites to treatments with thrips did not result in an increased predator population compared to a treatment with only thrips. Maybe spider mite densities were too low for such an effect to occur. We suggest that both the higher availability of prey and the effects of a mixed diet contributed to a high predator population. These high predator densities, in turn, contributed to improved control of spider mites. Although *A. swirskii* is not an efficient spider mite predator, it nevertheless reduced spider mite damage when the predator-prey ratio was sufficiently high.

In summary, we provide evidence that diversity of pest species enhances biological control of whiteflies and spider mites with a generalist predatory mite. Similar effects might also be achieved by adding a non-pest alternative food source, such as pollen. Several studies have shown the benefit of pollen in terms of enhancing pest control (Nomikou et al., 2002, 2010; van Rijn et al., 2002), but so far, this has not resulted in large-scale applications. Our results furthermore suggest that it might be advantageous to allow or create some pest species diversity in a crop, rather than to try to exterminate all phytophages present. Further experiments have to be done to determine whether the total crop damage of three or two pest species is less than the damage inflicted by one of the pest species. Releasing pest species in a crop is considered risky, but is accepted in some cases, such as in sweet pepper, where some growers use the 'pest-in-first' strategy with spider mites to enhance control by *P. persimilis* (Hussey et al., 1965; K. Bolckmans, personal communication). Avoiding total eradication of all pest species, thereby maintaining some pest diversity, might be more acceptable for the growers than introducing a new pest. For example, for cotton is has been suggested to leave a 'pest residue' as food for predators early in the season in order to enhance biological control of pests that occur later in the season (Luckmann & Metcalf, 1975; Gonzalez & Wilson, 1982). The demonstrated effects of pest diversity on a generalist predator in this study might furthermore be useful for evaluation programs of 'new' generalist predators by assessing their performance not only on the target prey alone, but also in the presence of other relevant pest species.

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Hyperpredation by generalist predatory mites disrupts biological control of aphids by the aphidophagous gall midge *Aphidoletes aphidimyza*

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Biological control of different species of pest with various species of generalist predators can potentially disrupt the control of pests through predator-predator interactions. We evaluate the impact of three species of generalist predatory mites on the biological control of green peach aphids, *Myzus persicae* (Sulzer) with the aphidophagous gall midge *Aphidoletes aphidimyza* (Rondani). The predatory mites tested were *Neoseiulus cucumeris* (Oudemans), *Iphiseius degenerans* (Berlese) and *Amblyseius swirskii* Athias-Henriot, which are all commonly used for pest control in greenhouse sweet pepper. All three species of predatory mites were found to feed on eggs of *A. aphidimyza*, even in the presence of abundant sweet pepper pollen, an alternative food source for the predatory mites. In a greenhouse experiment on sweet pepper, all three predators significantly reduced population densities of *A. aphidimyza*, but aphid densities only increased significantly in the presence of *A. swirskii* when compared to the treatment with *A. aphidimyza* only. This stronger effect of *A. swirskii* can be explained by the higher population densities that this predator reached on sweet pepper plants compared to the other two predator species. An additional experiment showed that female predatory midges do not avoid oviposition sites with the predator *A. swirskii*. On the contrary, they even deposited more eggs on plants with predatory mites than on plants without. Hence, this study shows that disruption of aphid control by predatory mites is a realistic scenario in sweet pepper, and needs to be considered when optimizing biological control strategies.

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Biological control of a particular pest species is increasingly becoming embedded in a community of multiple species of natural enemies and pests, which interact in several direct and indirect ways (Sih et al., 1985; Janssen et al., 1998; Prasad & Snyder, 2006; Evans, 2008). Especially generalist predators that feed on multiple prey and on other predators may negatively affect biological control (Symondson et al., 2002). One widely studied interaction is intraguild predation, which occurs when one predator species (the intraguild predator) kills and eats another predator species (the intraguild prey) with whom it also competes for shared prey (Polis et al., 1989; Holt &

Polis, 1997). In theory, intraguild predation can disrupt biological control (Rosenheim et al., 1995), but in practice, results are mixed (Janssen et al., 2006, 2007; Vance-Chalcraft et al., 2007).

Predators can also attack other predators without sharing a prey, with each predator feeding on a different prey species, so-called hyperpredation (see Müller & Brodeur, 2002). However, the literature is not univocal on the terminology for this type of interaction. Some prefer to use the term ‘secondary predation’ (Rosenheim et al., 1995), or the more general term ‘higher-order predation’ (Rosenheim, 1998; Symondson, 2002) for predators consuming other predators, which includes both hyperpredation and intraguild predation. In conservation biology some predator-mediated prey-prey interactions are described as hyperpredation (e.g., Courchamp et al., 2000), whereas it would be more consistent to refer to these interactions as apparent competition (Holt, 1977). For our study system we prefer to use hyperpredation for predators eating other predators without sharing a prey, because the type of interaction is similar to hyperparasitism. Hyperpredation seems to be less documented than intraguild predation, but it has been reported to weaken pest suppression in some cases (Snyder & Ives, 2001; Rosenheim, 2001; Kaplan & Eubanks, 2002; Prasad & Snyder 2004; Rosenheim et al., 2004). So far, no specific theory has been proposed about the effects of hyperpredation by generalist predators on prey populations. In contrast, the effects of hyperparasitism have been described, both theoretically (Beddington & Hammond, 1977; May & Hassell, 1981) as well as empirically (Sullivan & Völkl, 1999). These studies indicate that obligate hyperparasitoids always lead to an increase of the pest equilibria, which might be detrimental to biological control.

Several factors might relax the effects of generalist predators on other natural enemies, such as anti-predator behaviour, habitat structure, habitat specialisation, spatial heterogeneity and alternative prey (Krivan, 2000; Heithaus, 2001; Janssen et al., 2006, 2007; Daugherty et al., 2007; Holt & Huxel, 2007; Sabelis et al., 2009). Hence, empirical studies on the interaction among predators that include such factors are needed for assessing the effects on biological control.

In this study, we examined the interactions between generalist predatory mites and the aphidophageous gall midge *Aphidoletes aphidimyza* (Rondani) in greenhouse sweet pepper plants. This predatory midge is regularly used for control of aphids in greenhouses, because the larvae are effective predators of several aphid species and the adults are very efficient at locating aphid colonies (Markkula et al., 1979; Blümel, 2004; Choi et al., 2004). Generalist phytoseiid predatory mites are used for controlling other major greenhouse pest species such as thrips, whiteflies and spider mites (Gerson & Weintraub, 2007; Sabelis et al., 2008). In sweet pepper, populations of these predators can be established even in the absence of prey, because the continuous production of pollen provides sufficient food for the predators (Shipp & Ramakers, 2004). We used the predatory mites *Neoseiulus cucumeris* (Oudemans), *Iphiseius degenerans* (Berlese) and *Amblyseius swirskii* Athias-Henriot, which are all commonly used for control of thrips in sweet pepper (Shipp & Ramakers, 2004; Bolckmans et al., 2005; Gerson & Weintraub, 2007). Moreover, *A. swirskii* is used for whitefly control in sweet pepper (Bolckmans et al., 2005). These generalist predatory mites can be classified as hyperpredators, because they can feed on eggs of *A. aphidimyza* (van Schelt & Mulder, 2000; Messelink et al., 2005) but not on aphids (see below). For assessing the effects of predatory mites on *A. aphidimyza* and the suppression of aphids, we specifically address the following questions: (1) What are the predation rates of generalist predatory mites on *A. aphidimyza* eggs? (2) Does pollen, an alternative food source for the predatory mites, affect these predation rates? (3) What are the consequences of these interactions for the suppression of aphids? (4) Does *A. aphidimyza* avoid hyperpredation through selection of enemy-free aphid colonies? The prey in our experiments was the green peach aphid, *Myzus persicae* (Sulzer), a major pest species in greenhouse vegetables (Blümel, 2004). By answering the above four questions, we aim to better understand and predict the compatibility of important predators of thrips, whiteflies and aphids in greenhouse crops.

Materials and Methods

Rearing

Sweet pepper plants, *Capsicum annuum* L. cv. Ferrari (Enza Seeds, Enkhuizen, The Netherlands), were grown in rockwool blocks in a greenhouse compartment. We used the red phenotype (Gillespie et al., 2009) of the green peach aphid, *M. persicae*, which was cultured on sweet pepper plants. The predatory mites used for assessing predation rates were all reared on pollen. *Iphiseius degenerans* was reared on flowering (pollen producing) castor bean *Ricinus communis* L. in a greenhouse compartment. *Amblyseius swirskii* and *N. cucumeris* were reared in climate rooms on cattail pollen, *Typha latifolia* L., on sweet pepper leaves placed upside down on water-saturated cotton wool in small plastic boxes, with 16 h of artificial illumination per day, at 22°C and 70% RH. The predatory midge *A. aphidimyza* and the predatory mites for greenhouse releases of *A. swirskii* were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands).

Predation on aphids by predatory mites

We verified the assumption that the predatory mites in this study do not feed on aphids by observing their behaviour in the presence of first instar aphids. Females of *N. cucumeris*, *I. degenerans* and *A. swirskii* were starved for 24 h to ensure that all predatory mites were motivated to feed. Sweet pepper leaf discs (4 cm diameter) were put upside down on wet cotton and infested with 4 reproducing females and 10-15 first instars of *M. persicae*. For each predatory mite species, we observed 20 starved females for 5 minutes with a binocular microscope (40×). Each individual predatory mite was put on a separate leaf disc with aphids. The number of encounters with a visible response of the aphids (kicking a leg) and successful attacks (killing of aphids) was recorded per mite. After these 5 minute observations, the mites were left on the leaf discs for 24 h, after which leaf discs were checked for the presence of killed aphids. The observational data were analysed using a generalized linear model with a Poisson distribution and predator species as factor. Differences were determined to be significant at $p<0.05$.

Additionally we tested whether population increases of aphids could be affected by predatory mites. For this, we used the species *A. swirskii* only (most active in the observational experiment). Eight leaf discs were embedded upside-down in water agar (1% agar), each in a separate plastic box (5 cm high, diameter 6 cm), making the abaxial side of the discs available to the aphids. To each box we added 10 2-day-old females of *M. persicae*, which started to produce juvenile aphids shortly after putting them on the leaf discs. Ten one-week-old females of *A. swirskii* were added to half of these boxes (10 per 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. Boxes were incubated at 16 h of artificial illumination per day, 22°C and 70% RH. Aphid and mite densities were assessed after 3 and 7 days. Aphid densities were analysed with a repeated measures ANOVA, performed on $\log(x+1)$ transformed numbers.

Predation on midge eggs by predatory mites

Predation on eggs of *A. aphidimyza* was measured for the three predatory mite species *A. swirskii*, *N. cucumeris* and *I. degenerans* on glass arenas (20 × 20 mm) in the absence or presence of an ample supply of sweet pepper pollen. The arenas were placed on water-saturated cotton wool in plastic boxes and the edges of the arenas were covered with strips of wet tissue paper in order to supply the mites with water (according to van Rijn & Tanigoshi 1999). Each arena was supplied with 12 *A. aphidimyza* eggs, collected with a fine brush from an aphid-colonized sweet pepper leaf that was exposed to ovipositing females of *A. aphidimyza* in a cage during one day. In addition to *A. aphidimyza* eggs, 20 µg of sweet pepper pollen was added to half of the treatments. The pollen was collected from sweet pepper flowers (cv. Ferrari) and stored in a freezer for about 1 month. It is known to be a good food source for predatory mites (Vantornhout et al., 2004). Single mated female predatory mites (1 week old) were starved for 24h to ensure that all predatory mites were motivated to feed, and were subsequently placed on the glass arenas for measuring predation rates. The experiment was performed in a climate room at 22°C, 70% RH and 16 h of artificial illumination per day. After 24 h, we counted the number of preyed *A. aphidimyza* eggs (egg content removed). Each mite-food combination was replicated 12×. Replicates where mites ran into the water were excluded from data analyses and these replicates were repeated with other mite individuals.

Predation rates of predatory mites in the absence of pollen were analysed using a generalized linear model with a Poisson distribution and predator species as factor. The effect of adding pollen was analysed for each predator species with the same models, but now with the presence or absence of pollen as factor. Differences were determined to be significant at $p<0.05$.

Preliminary observations of predatory mites in the presence of aphids and larvae of *A. aphidimyza* showed that predatory mites only incidentally attacked midge larvae. They seem not able to prey on them because the midge larvae defend themselves with rapid head movements towards the attacking predatory mites. To verify this, we added predatory mites of *A. swirskii* to boxes with aphids and midge larvae. Eight plastic boxes were supplied with agar and sweet pepper leaf discs as described above (section 'Predation on aphids by predatory mites'). To each box we added 80-100 aphids of mixed age. The boxes were placed upside down on a tray of gauze in a cage with adults of *A. aphidimyza* for 1 day, in order to allow them to oviposit near the aphids. The boxes were removed after one day and incubated in a climate chamber as described above. After 3 days, we counted the number of midge larvae per box and removed all unhatched eggs so that only midge larvae were present. The number of larvae varied from 30-60 per box. Subsequently, we added four adult female predatory mites of *A. swirskii* to each box and placed the boxes in the same climate chamber. Numbers of midge larvae and mites were counted again after 1 and 3 days.

Effects of hyperpredation on aphid suppression

We conducted a greenhouse experiment in spring-summer to investigate the impact of three species of predatory mites on aphid control by the predatory midge *A. aphidimyza* in a sweet pepper crop. Sweet pepper plants, cv. Ferrari, were planted in a loamy soil in two greenhouse compartments of 96 m^2 each. The experimental unit was one group of four sweet pepper plants enclosed in a walk-in-cage of 1 × 2 × 2 m. In total we used 20 cages. Each plant was grown according to a three-stems-per-plant system, so in total there were 12 sweet pepper stems per cage. The experiment had a randomized block design with four replicates of the following treatments: (1) aphids, (2) aphids + *A. aphidimyza*, (3) aphids + *A. aphidimyza* + *A. swirskii*, (4) aphids + *A. aphidimyza* + *N. cucumeris* and (5) aphids + *A. aphidimyza* + *I. degenerans*.

The predatory mites were released before the aphids and *A. aphidimyza* to mimic the common greenhouse practice of releasing predatory mites on young plants. Aphid infestations commonly occur later in the season, when predatory mites have already established. The first predator introductions started 5 weeks after planting, when the plants were about 1 m high and flowering, thus supplying pollen. The predatory mites were released at densities of 100 individuals of mixed age/plant and this was repeated after 3 weeks in order to ensure the establishment of predator populations. Two weeks after the first predatory mite introductions, we infested plants with the aphid *M. persicae* at densities of 10 aphids of mixed age per stem, thus 30 aphids per plant. This was done by transferring the aphids from a culture on sweet pepper to upper leaf layers with a fine paintbrush. The aphidophagous midge was released 3 and 4 weeks after the aphid introduction through adding 200 pupae (sex ratio 50%) in a humid layer of vermiculite. The interval between aphid introduction and releases of predatory midges enabled the aphids to establish and increase in population density. The first adults of *A. aphidimyza* emerged 4 days after these introductions. Densities of aphids and predators were assessed weekly for 4 weeks, starting 3 weeks after the aphid introduction (thus the first assessment was without predatory midges). This was done by randomly picking 10 leaves per cage from the upper 50 cm of the plant and transporting these leaves to the laboratory, where they were observed using a binocular microscope. Predatory mites were mounted on slides for further identification. Temperature and relative humidity were registered every 5 minutes in one cage of each greenhouse compartment throughout the experiment with a climate recorder. The values were nearly equal in the two greenhouses, with average temperatures of 22.4 and 22.8°C and average relative humidities of 66 and 68%. For statistical analyses, a repeated measures ANOVA was performed on log(x+1) transformed numbers of aphids, midge eggs, midge larvae and predatory mites with the time since introduction of aphids as the repeated measure variable. Differences among treatments with or without predators were tested at a 5% level using Fisher's LSD.

Oviposition behaviour of *Aphidoletes aphidimyza*

To test whether the presence of predatory mites on plants affected the oviposition behaviour of *A. aphidimyza*, a short greenhouse experiment was conducted with isolated pepper plants placed in a circle. Twelve sweet pepper plants, cv. Ferrari, were placed in a circle with a diameter of 3 m in a 24 m^2 greenhouse compartment. The plants were 2 months old and had on average 40 leaves per plant. Each plant was isolated and did not touch other plants. Furthermore, contamination was prevented among the plants with a water barrier by placing each plant in a plastic pot on a dish with water. Plants were infested with *M. persicae* by transferring a total of 40 individuals of mixed age with a fine paintbrush to four leaves of each plant. Half of the plants were alternately infested with the predatory mite *A. swirskii* by adding 200 individuals in the carrier bran near the plant base, 5 days after the aphid releases. This predator release rate corresponds with an average density of five mites per leaf, which is common for *A. swirskii* (Calvo et al., 2009). One-day-old adults of *A. aphidimyza* were released 5 and 6 days after the aphid introduction in the middle of the circle of plants. In total we released 371 adults (51% female): 194 on day 5 and 177 on day 6. These adults had access to droplets of honey during the first day of their adult lives.

Because the adults of *A. aphidimyza* are active at night, we were unable to observe their behaviour. Instead, we observed the oviposition behaviour indirectly by counting the number of midge eggs and larvae per plant. Note that predation on midge eggs by the predatory mites could have affected these observations. To minimize this effect, we released high numbers of midges and made our observations in the early morning, thus shortly after the night when females lay their eggs. Numbers of aphids, midge eggs and midge larvae per plant were counted daily with a head-worn binocular loupe (Zeiss KF 5x) over a period of 4 days, starting from the day after the first releases of the midges. One final assessment was done after 7 days, including a count of the predatory mites. The average greenhouse temperature was 22.0°C and the average relative humidity 72%. Data of the first four daily observations were analyzed with a repeated measures ANOVA, performed on log(x+1) transformed numbers of aphids and midge eggs. The data of the final assessment were analyzed using a one-way ANOVA with log(x+1) transformed numbers of aphids, midge eggs and midge larvae as response variables. Differences between treatments with or without predators were tested at a 5% level using Fisher's LSD method.

Results

Predation on aphids by predatory mites

The average number of encounters in which aphids responded to predatory mites by leg kicking varied between 1.0 and 1.4 and did not differ significantly among the mite species ($F_{2,57} = 0.78$, $p = 0.46$). These encounters never resulted in a successful attack of the aphid, neither within the 5 minutes of observation, nor after 24 h. In both treatments, aphid densities increased from 10 to ca. 190 individuals per disc within 1 week, and the difference between treatments was not significant ($F_{1,6} = 0.23$, $p = 0.65$). Survival of the predatory mites was low (30% mortality) on leaf discs with aphids as the only available food source, whereas females of the same age fed with pollen survived and reproduced. Thus, both experiments confirm that these phytoseiids do not directly affect aphid densities, either by killing or through disruption of aphid behaviour.

Predation on midge eggs by predatory mites

Predation on *A. aphidimyza* eggs in the absence of pollen did not differ significantly among the three tested predatory mite species ($F_{2,33}$ = 1.56, p = 0.23). Starved female predatory mites consumed on average 6-8 eggs per day (Figure 6.1). This predation resulted in the predatory mites turning red, especially in the case of *A. swirskii* and *N. cucumeris* that commonly appear more yellowish-brown. The presence of sweet pepper pollen did not significantly affect these predation rates for *A. swirskii* ($F_{1,22}$ = 1.26, p = 0.27) and *N. cucumeris* ($F_{1,22}$ = 2.52, p = 0.13), but pollen significantly reduced the predation rates for *I. degenerans* ($F_{1,22}$ = 8.86, p = 0.007). The midge larvae were not consumed by *A. swirskii*. Although all *A. swirskii* survived in the presence of aphids and midge larvae, not a single mite showed the typical red colouring which appears after feeding on the red midge larvae.

Effects of hyperpredation on aphid suppression

Aphid populations increased rapidly to high densities of hundreds per leaf up to the fifth week in all treatments (Figure 6.2). Treatments significantly affected the aphid population dynamics ($F_{4,15}$ = 7.11, p = 0.002). Out of the three tested predatory mite species, only the addition of *A. swirskii* to *A. aphidimyza* resulted in significantly higher levels of aphids compared to the treatment with *A. aphidimyza* only (Figure 6.2). The numbers of *A. aphidimyza* eggs across time were significantly lower in the presence of predatory mites *A. swirskii* and *I. degenerans* ($F_{3,12}$ = 10.63, p = 0.001) (Figure 6.3A). The numbers of *A. aphidimyza* larvae through time were significantly lower in the presence of any of the three species of predatory mites, with the lowest densities in the treatment with *A. swirskii* ($F_{3,12}$ = 8.66, p = 0.002) (Figure 6.3B).

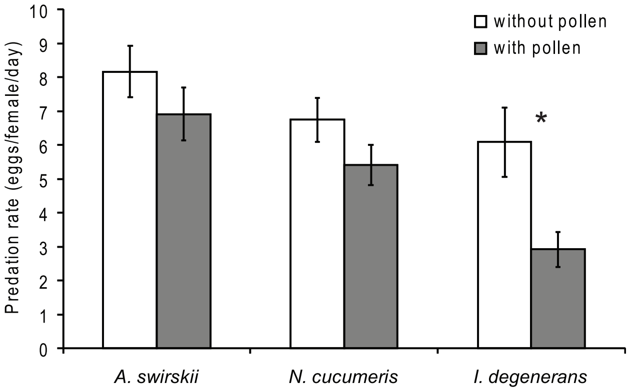

Figure 6.1 – Rates of predation on eggs of the predatory midge *A. aphidimyza* by adult females of three species of predatory mites in the absence or presence of sweet pepper pollen. Asterisk (*) indicates a significant effect of the presence of pollen on predation rates (p<0.05).

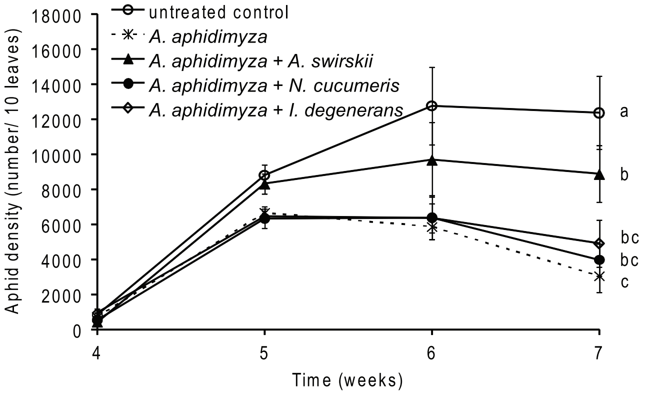

FIGURE 6.2 – Population dynamics of aphids (*M. persicae*) in a sweet pepper crop in the absence or presence of the predatory midge *A. aphidimyza* and in the presence of *A. aphidimyza* + generalist predatory mites (*A. swirskii*, *N. cucumeris* or *I. degenerans*). Shown are average (± SE) aphid densities per 10 leaves. Aphids were introduced in the first week and *A. aphidimyza* in the third and fourth week. Predators were released prior to aphid releases. Different letters indicate significant differences among treatments through time (Fisher's LSD test, p<0.05).

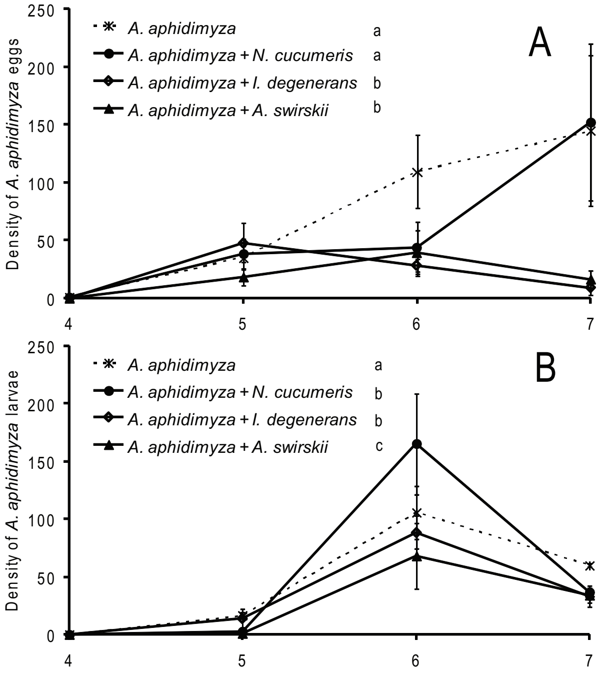

FIGURE 6.3 – Average densities (± SE) of eggs (A) and larvae (B) of the predatory midge *A. aphidimyza* per 10 leaves in a sweet pepper crop in the absence or presence of generalist predatory mites (*A. swirskii*, *N. cucumeris* or *I. degenerans*). See legend to FIGURE 6.2 for further explanation. Different letters indicate significant differences among treatments through time (Fisher's LSD test, p<0.05).

Densities of the predatory mites differed significantly among treatments ($F_{2,9} = 20.29$, $p<0.001$), with *A. swirskii* having the highest densities (7.3/leaf), followed by *I. degenerans* (2.7/leaf) and *N. cucumeris* (1.3/leaf) respectively. In all treatments, we observed low densities of spontaneously occurring Western flower thrips, *Frankliniella occidentalis* (Pergande) (on average between 0 and 1 larva/leaf in week 4 to between 1 and 3 larvae/leaf in week 7). We never observed predation on *A. aphidimyza* by thrips during leaf assessments, neither did we observe red coloured individuals of thrips, suggesting that thrips do not prey on this predatory midge. Other spontaneously occurring pest species were not detected.

Oviposition behaviour of *Aphidoletes aphidimyza*

Densities of aphids did not differ significantly between plants with or without predatory mites during the first 4 days ($F_{1,10} = 0.64$, $p = 0.44$; FIGURE 6.4A). Thus, oviposition preferences of *A. aphidimyza* could not have been affected by aphid densities during this period. Densities of midge eggs were also not significantly different between the two treatments during the first 4 days ($F_{1,10} = 1.22$, $p = 0.30$), but there was a trend of lower midge densities on the plants treated with predatory mites dur-

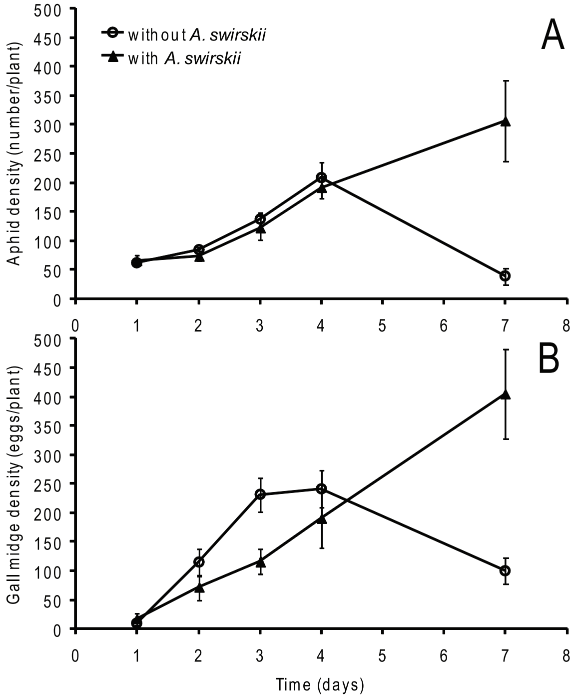

FIGURE 6.4 – Population dynamics of aphids (A) and eggs of the predatory midge *A. aphidimyza* (B) on sweet pepper plants with or without the predatory mite *A. swirskii*. Shown are average densities (± SE) per plant.

ing these first 4 days (FIGURE 6.4B). On day 7, we found significant differences between treatments for both aphid and midge densities (FIGURES 6.4 and 6.5). Densities of aphids were lower on plants with predatory midges only than on plants treated with predatory mites and predatory midges ($F_{1,10}$ = 11.68, p = 0.007), whereas the opposite was found for midge larvae: significantly lower densities on plants with predatory mites than on the control plants without predatory mites ($F_{1,10}$ = 22.03, p<0.001). Densities of midge eggs were significantly higher on plants with predatory mites than on the plants without predatory mites ($F_{1,10}$ = 24.93, p<0.001. Predatory mites were found all over the plants in the predator treatments (average densities of 164/plant (SE = 28.2), including the leaves with aphids. Control plants did not harbour any predatory mites. The results of this experiment suggest that females of *A. aphidimyza* strongly prefer to oviposit on plants with higher aphid densities and do not avoid plants with predatory mites.

Discussion

Several studies have shown that predators can attack and kill other natural enemies, but so far, few studies have shown the impact on pest suppression (Janssen et al., 2006, 2007; Vance-Chalcraft et al., 2007). Our results demonstrate that hyperpredation of a specialist aphid predator by generalist predatory mites can disrupt the biological control of aphids.

We found that the three species of predatory mite all fed on eggs of the aphidophagous gall midge *A. aphidimyza*, which is in agreement with earlier observations (van Schelt & Mulder, 2000; Messelink et al., 2005). The presence of pollen, which is

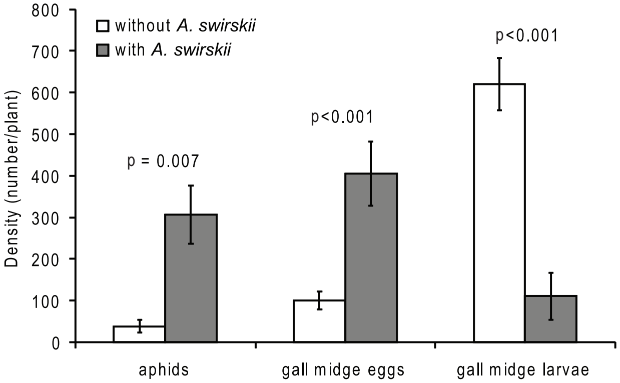

FIGURE 6.5 – Densities of aphids and eggs and larvae of the predatory midge *A. aphidimyza* on plants with or without the predatory mite *A. swirskii*. Shown are the average densities (± SE) 7 days after the first releases of midge adults. The p-values refer to the significance of differences between treatments per organism, based on Fisher's LSD test.

common in a sweet pepper crop, only slightly reduced the predation rates of *A. swirskii* and *N. cucumeris*. However, the presence of pollen did significantly reduce predation rate on *A. aphidimyza* eggs by *I. degenerans*. Similar results were found in other studies concerning pollen-prey combinations with *A. swirskii* and *I. degenerans* (Palevsky et al., 2003; Nomikou et al., 2004). Other preference studies with *A. swirskii* and *N. cucumeris* showed that these mites preferred prey with the highest quality in terms of reproduction (Buitenhuis et al., 2010). Such preferences might have played a role in our study as well. However, we did not measure prey quality in terms of reproduction as this was beyond the scope of our study.

On sweet pepper plants in greenhouses, we demonstrated the risks of using predatory mites and *A. aphidimyza* in one crop. All three predators significantly reduced populations of *A. aphidimyza*, but only *A. swirskii* significantly affected the population dynamics of aphids, resulting in 3x higher aphid densities compared to plants with only *A. aphidimyza*. The predators *N. cucumeris* and *I. degenerans* suppressed *A. aphidimyza* densities less than *A. swirskii*, which might be explained by the lower densities of these mites (1 and 3 mites/leaf respectively) in the crop, compared to *A. swirskii* (7 mites/leaf). The releases of the predatory mites prior to the aphids and midges resulted in different population densities of predatory mites before the midges were introduced. Consequently, we were not able to compare the effects on aphid control among the three species of predatory mite at equal predator densities, but this, on the other hand, allowed us to assess the predator effects under a common scenario in a sweet pepper crop. Other studies report similar differences in density among these predators on pepper plants in the presence of thrips (Bolckmans et al., 2005; Arthurs et al., 2009). The differences in our study might be the result of their performance on a mixture of food present on the plants, such as *A. aphidimyza* eggs, aphid honeydew, pollen, nectar and some larvae of thrips. Remarkably, the reduced numbers of *A. aphidimyza* larvae through predation by *I. degenerans* and *N. cucumeris* did not result in higher populations of aphids. Midge larvae need at least seven aphids (in the case of *M. persicae*) to complete their larval development, but kill on average 25 aphids at high aphid densities, and thus do not consume the entire content of the aphids (Uygun, 1971). Aphid densities in our experiment were on average high compared to the densities of midge larvae. Hence, increased attack rates and partial ingestion may explain the absence of an indirect effect of *I. degenerans* and *N. cucumeris* on aphid densities. In addition to direct predation effects of predatory mites, some trait-mediated effects might have affected aphid densities as well (Prasad & Snyder, 2006). For example, it could be that the midge larvae were disturbed by the predatory mites, which consequently could affect the midge-aphid interaction. The presence of thrips might also have affected the aphids directly or indirectly, but because of the relatively low numbers of thrips this was not likely to be a strong effect.

In a study on potato, Lucas & Brodeur (1999) showed that *A. aphidimyza* females preferred to oviposit on sites with high trichome densities, where the risk of predation by coccinellid intraguild predators was reduced (Lucas & Brodeur, 1999). Thus, it is possible that *A. aphidimyza* females select oviposition sites with lower risks of predation. We therefore assessed whether ovipositing females of *A. aphidimyza* avoided predation by predatory mites through selection of enemy-free aphid colonies. However, we found no such avoidance. At equal aphid densities, no significant differences were found in the densities of *A. aphidimyza* eggs. This suggests that female midges ignore the presence of predatory mites when they oviposit near aphid colonies. After 7 days, we even saw the opposite of anti-predator behaviour; more eggs were deposited on plants with predatory mites than on plants without these predators. This last phenomenon can be explained by the strong preference of female midges to oviposit on sites with higher aphid densities (El Titi, 1973; Lucas & Brodeur, 1999; Choi et al., 2004). Thus, the results suggest that the predatory mite *A. swirskii* only indirectly affects the oviposition behaviour of the female midges through predation on midge eggs. This predation resulted in 6× lower densities of midge larvae, which is the stage responsible for aphid consumption. Consequently, aphid densities on plants with predatory mites were 8× higher than on plants without predators. These higher aphid densities finally caused midges to oviposit more on plants with the predatory mite *A. swirskii*.

In summary, generalist predatory mites can disrupt biological control of aphids with the predatory midge *A. aphidimyza*. This hyperpredation emphasizes the importance of an entire-ecosystem view when designing biological control strategies for multiple pest species. The predatory mite *A. swirskii* was the most disrupting for aphid control, but this predator is very important for the control of whiteflies, thrips, spider mites and broad mites (Nomikou et al., 2002; Messelink et al., 2006, 2010; Arthurs et al., 2009; Calvo et al., 2009; van Maanen et al., 2010). Greenhouse crops, fortunately, offer the unique possibility to create the desired community of natural enemies by choosing and releasing the necessary natural enemies from the many species commercially available (van Lenteren, 2000; Enkegaard & Brødsgaard, 2006). Thus, based on the abundance, diversity and potential risk of pest species, it is possible to adapt the strategies of natural enemy releases. For example, in organic greenhouse production systems of sweet pepper in The Netherlands, aphids are much more serious pests than thrips. In such cropping systems, it might be better to use thrips predators that are more compatible with specialised aphid enemies. Generalist predatory bugs that feed both on thrips and aphids (e.g., *Orius* spp.) might be a good alternative for predatory mites, but intraguild predation by such predators is also a potential risk (Christensen et al., 2002; Hosseini et al., 2010). We suggest that more experiments are needed to evaluate multiple pest control with diverse assemblages of natural enemies, because essential information about species interactions within these communities is still lacking. This is not only important for further development of effective biological control strategies, but can also be used for testing and extending theories on multispecies interactions.

Acknowledgements
We thank E. de Groot, W. van Wensveen, L. Kok and R. van Holstein-Saj for assistance in the greenhouse experiments. This study was funded by the Dutch Ministry of Agriculture, Nature and Food Quality. Koppert Biological Systems is thanked for supplying the natural enemies. Comments by two anonymous reviewers substantially improved the manuscript.

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

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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.

Submitted for publication

Generalist 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 *Ephestia 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^2 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.*

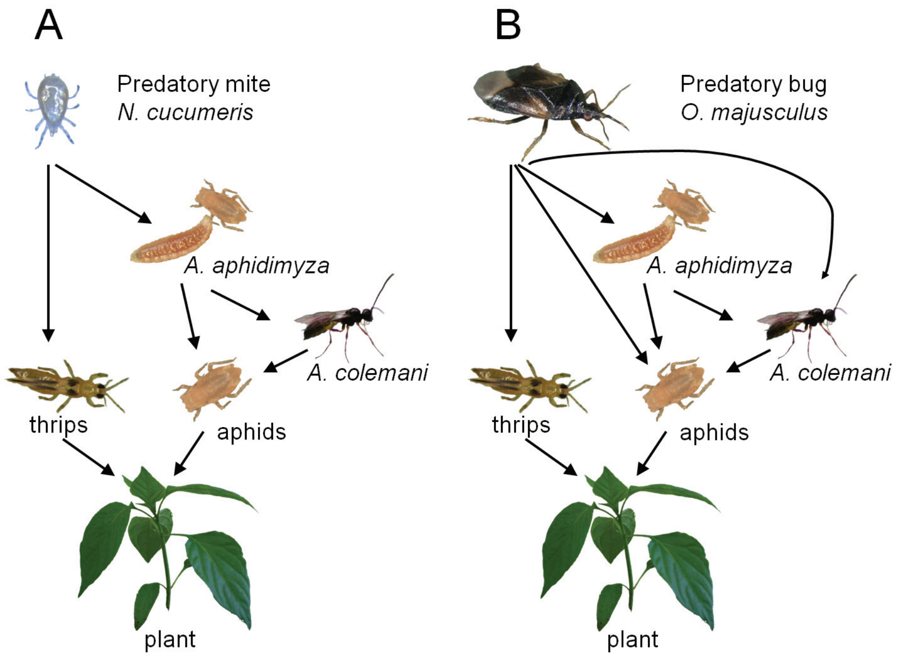

FIGURE 7.1 – Two strategies for biological control of thrips and aphids in sweet pepper. Arrows indicate consumption of the species at the tip of the arrow by the species at the base of the arrow. Strategy A involves hyperpredation of aphid predatory midges by predatory mites, whereas strategy B involves 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								
Neoseiulus cucumeris[1]	1800							
Orius majusculus[2]	100	100						
PEST SPECIES								
Myzus persicae[1]			36	72	144			
Frankliniella occidentalis[3]			36	36				
APHID ENEMIES								
Aphidoletes aphidimyza[4]					10	20	20	100
Aphidius colemani[4]					6	10	10	20

[1]mixture of juveniles and adults; [2]released as adults, 60% female; [3]adult females; [4]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 followed 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 (± SE) temperatures of 21.2 ± 0.04°C and average relative humidities of 71 ± 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 illumination 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.

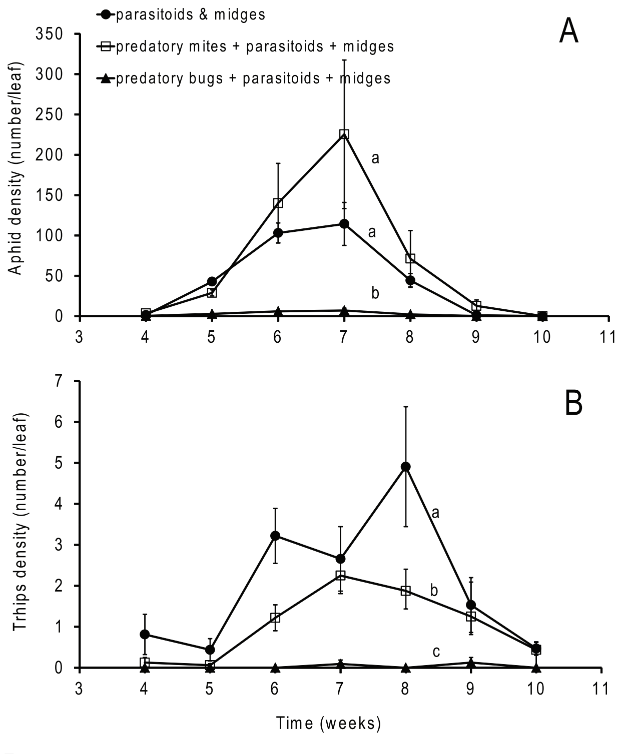

FIGURE 7.2 – Population dynamics of (A) the green peach aphid *Myzus persicae* and (B) Western flower thrips *Frankliniella occidentalis* in a sweet pepper crop in the presence of three assemblages of natural enemies. All three treatments received parasitoids (*Aphidius colemani*) plus predatory midges (*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 (± SE) densities per leaf. Different letters indicate significant differences among treatments through time (Fisher's LSD test, $p<0.05$).

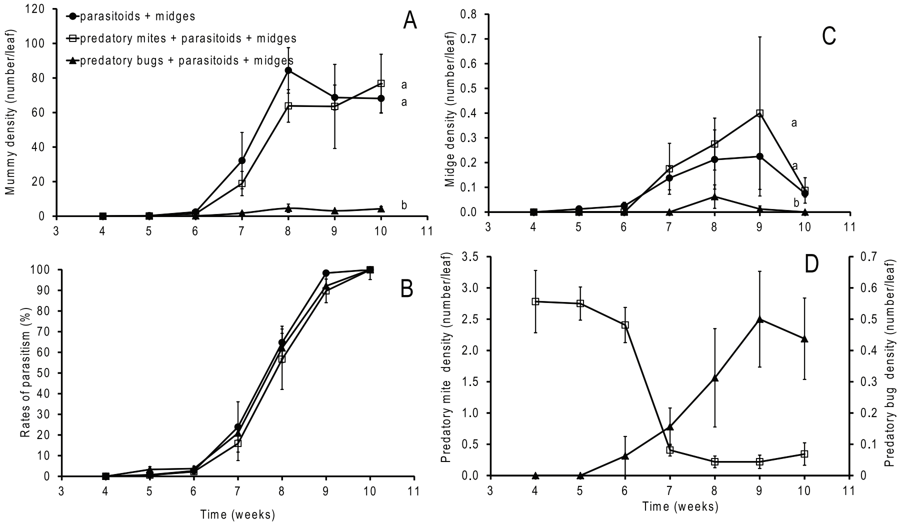

FIGURE 7.3 – Population dynamics of (A, B) the parasitoid *Aphidius colemani*, (C) the predatory midge *Aphidoletes aphidimyza* and (D) the predatory mite *Neoseiulus cucumeris* and the predatory bug *Orius majusculus* in a sweet pepper crop infested by the green peach aphid *Myzus persicae* and Western flower thrips *Frankliniella occidentalis*. See legend to FIGURE 7.2 for further explanation. Shown are average (± SE) percentages of parasitized aphids and average (± 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).

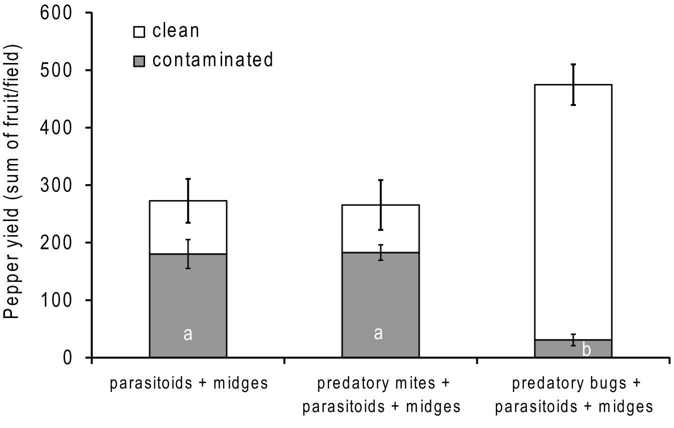

FIGURE 7.4 – Total number (± SE) of clean and honeydew-contaminated pepper fruits from plants infested with the green peach aphid *Myzus persicae* and Western flower thrips *Frankliniella occidentalis* in the presence of three assemblages of natural enemies. Fruit production was measured during 18 weeks. 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).

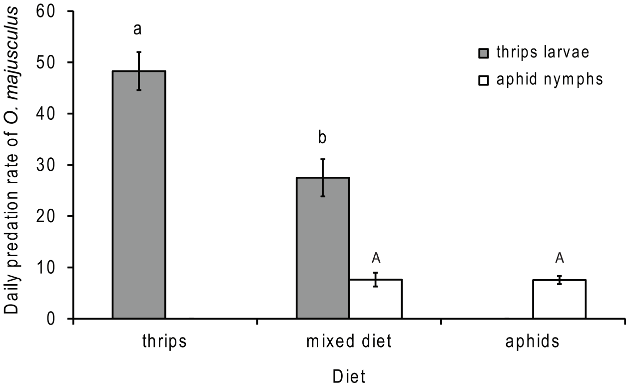

FIGURE 7.5 – Number of prey consumed by one-week-old adult females of *Orius majusculus* per day when offered second instar thrips larvae and third instar aphid nymphs either separately or in combination (mixed diet). Shown are average numbers of prey consumed (± SE) per female per day (measured over 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).

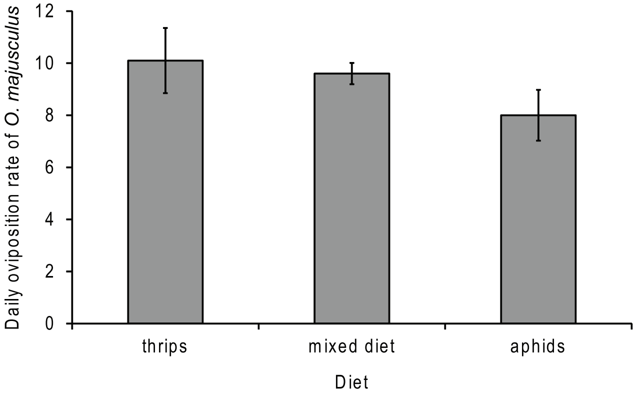

FIGURE 7.6 – Average daily oviposition rates of 10-days-old adult female *Orius majusculus* when offered either thrips larvae and aphid nymphs separately or in combination (mixed diet). Shown are average numbers of eggs (± SE) per female per day.

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 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.

Acknowledgements

This study was funded by the Dutch Ministry of Agriculture, Nature and Food Quality. We thank L. Kok and E. de Groot for assistance in the greenhouse and laboratory experiments. Koppert Biological Systems and Biobest NV are thanked for supplying the natural enemies. Comments by two anonymous reviewers and Patrick De Clercq substantially improved the manuscript.

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8

General discussion

As emphasized in the introduction to this thesis, interactions between a pest and a natural enemy in agricultural systems are often embedded in complex food webs. The questions at the heart of this thesis are first, whether patterns expected from food web theory can be identified from the dynamics of arthropod communities in greenhouse crops (partly created through releases of natural enemies), and second, how interactions in these food webs affect the suppression of pest species. In this chapter I highlight and discuss the findings of this thesis.

Arthropod communities on plants often include generalist predators that feed on multiple prey. Compared to a situation with only one prey and one predator, theory on predator-mediated interactions between prey predicts lower equilibrium densities of the prey species in the long term, (apparent competition; Holt, 1977). I studied such indirect interactions between the pest species thrips, whiteflies and spider mites as a shared prey of generalist predatory mites. In CHAPTER 3, I show that these predators mediate apparent competition between thrips and whiteflies under non-equilibrium conditions. This effect was not reciprocal; only whiteflies were negatively affected by this predator-mediated interaction. Moreover, I present evidence that effects of apparent competition were strengthened by positive effects of a mixed diet of thrips and whiteflies on juvenile survival and developmental rate of the generalist predatory mites. Given that populations are well mixed and predators feed on both prey in the ratio in which they are encountered, a positive effect of mixed diets would result in a higher predator growth rate and consequently in an increase of equilibrium densities of the predator and a decrease of those of the prey.

The opposite effect of apparent competition may occur when increases in the density of one prey species result in satiation of the shared predators or in predator switching (when a predator eats disproportionately more of the most common type of prey), consequently reducing the consumption of the second prey species (Murdoch 1969; Abrams & Matsuda 1996). This effect is apparent in the short-term, when the densities have not yet reached an equilibrium (transient dynamics), because eventually, the predator populations will increase because of the higher densities of prey (Abrams & Matsuda 1996) and result in apparent competition. Apparent mutualism may also occur in the long term when population densities do not reach equilibria, but show cycles, resulting in repeated satiation of the shared predators and repeated reduced predation on the other prey (Abrams et al., 1998). In CHAPTER 4, I investigated if such population fluctuations of whiteflies result in long-term positive effects between whiteflies and thrips. In a first greenhouse experiment, population cycling was induced by releasing high densities of the two pest species at once. Because the larger juvenile stages and the adults of both pests are invulnerable to predation by the predatory mites, young stages that escape from predation due to predator satiation will reach adulthood and create a new generation of offspring. This in turn would result again in predator satiation, releasing thrips and whiteflies from control. The results of this experiment indeed showed that pest releases at once result in a high density of the second generation of whiteflies, which significantly delay the suppression of thrips populations. In a second experiment, this cycling was prevented by releasing the same densities of whiteflies as above, but spread over several weeks, resulting in a continuous presence of whitefly eggs as food for the predatory mites. This resulted in an opposite effect as was found in the first experiment: repeated releases of whiteflies had a negative effect on thrips populations compared to treatments in which no whiteflies were present. This was probably caused by a strong numerical response of the predators to the presence of prey stages that are suitable for consumption. Hence, I found that both positive and negative predator-mediated interactions between prey can occur, as predicted by the theory on these interactions (Holt, 1977; Abrams & Matsuda, 1996). To my best knowledge, this is the first study to show the occurrence of both these interactions within the time-scale of a single cropping cycle.

Theory on apparent competition concerns interactions between two prey species, but real communities may be more complex because predators feed on more than two prey species. In CHAPTER 5, I increased the food web complexity in cucumber by adding spider mites as a third pest species to the system of thrips, whiteflies and the generalist predator *Amblyseius swirskii*. The predator is not an effective predator of spider mites, because it is strongly hindered by the webbing produced by the spider mites. I show that not only whiteflies, but also spider mites are controlled better by the presence of thrips through apparent competition. Densities of yet another pest, spider mites, were even more suppressed when both thrips and whiteflies were present. This study points at another interesting aspect of apparent competition: generalist predators can have significant effects on prey species which they cannot suppress successfully in the absence of other prey. Such effects were, to my knowledge, not shown before in the literature on experiments testing for apparent competition.

Predator-mediated apparent competition may not only occur among herbivores, but also between herbivores and other natural enemies that are preyed upon by generalist predators. In CHAPTER 6, I demonstrate that generalist predatory mites used for the control of thrips, whiteflies and spider mites, also feed on eggs of the midge *Aphidoletes aphidimyza*, which is a predator of aphids. Because the predatory mites do not feed on aphids, I refer to this interaction as hyperpredation. As explained above, theory on apparent competition predicts that the presence of one prey lowers the equilibrium densities of the second prey. For hyperpredation, this would mean that increases in the densities of the prey of the hyperpredator will result in lower equilibrium densities of the specialist natural enemy, which would consequently release the prey of the specialist from control. I show that hyperpredation of predatory midges by predatory mites in the presence of thrips or pollen as food, indeed releases aphids from control. If hyperpredation depends on the density of both prey of the hyperpredator, than it is not immediately obvious how it will affect the dynamics of the two predator-prey systems. It might therefore be interesting to develop models on these kinds of interactions to further understand the possible long- and short-term effects. This is even more so if the two herbivores in this system may interact directly or through induced plant defences. In order to predict the extent to which hyperpredation affects pest control, it might be useful to study preferences of hyperpredators for the other natural enemy in comparison with the pest it should control.

Chapter 7 gives an example of the complexity of interactions in an arthropod community, where effects of one interaction may override the effects of another. I show that the possible release of aphids from control by predatory midges and parasitoids through intraguild predation by a generalist predatory bug was apparently outweighed by the direct negative effects of this predatory bug on aphids. Thus some effects that are potentially positive for prey species in food webs (such as intraguild predation) may be weak in comparison with other, negative effects of generalist predators.

Summarizing, the experiments presented in this thesis contribute to testing food web theory, specifically the theory of apparent competition. The results may contribute to a better understanding of the dynamics in complex food webs, and at the same time may help in developing biological control systems. One difficulty in comparing the experimental results with the existing theory is that agro-ecosystems often consider short-term (transient) dynamics, whereas theory is often based on equilibrium dynamics (Briggs & Borer, 2005). Theory and experiments on transient effects would be helpful for predicting and understanding species interactions in biological control systems. The findings in this thesis show that experiments in greenhouse ecosystems as model systems with relatively low species diversity and comparatively simple food webs can contribute to the evaluation and development of food web theory.

The questions that remain are how the interactions outlined above affect biological control and what this all means for the future of biological control in greenhouses. This thesis shows that both density-mediated interactions and behaviour-mediated interactions are common in greenhouse crops and affect the results of biological control. Especially the use of generalist predators will give rise to various types of interactions and to increased connectivity in food webs. Generalist predators were long considered as less effective than specialist natural enemies (Huffaker & Messenger, 1976; Hokkanen & Pimentel, 1984; van Lenteren & Woets, 1988; Hoy, 1994). Moreover, recent criteria for risk assessment of natural enemies consider the use of generalist predators as less desirable than specialist natural enemies (van Lenteren et al., 2006). However, the experiments in this thesis show effective control of thrips, whiteflies, spider mites and aphids by generalist predators (CHAPTERS 2, 3, 5 and 7). The evaluation of generalist predatory mites for thrips control in CHAPTER 2, together with the earlier results on whitefly control (Nomikou et al., 2002), were the reason for Koppert Biological Systems to start selling the predator *A.* (= *Typhlodromips*) *swirskii* on a commercial scale in 2005 (see www.allaboutswirskii.com). Nowadays, this predator is used in more than 20 countries and successfully applied in cucumber, sweet pepper, eggplant and some ornamental crops (Cock et al., 2010).

The role of generalist predators was recognized earlier by Murdoch et al. (1985), who argued that the biggest advantage of generalist predators is the persistence of populations (see also Chang & Kareiva, 1999; Symondson et al., 2002). In contrast, augmentative releases of specialist natural enemies often involve problems with timing, costs and quality of the natural enemies. The quality of specialist natural enemies depends on the host species on which they are reared and on the conditions during storage or transport (van Lenteren, 2003; Vasque & Baker, 2004). This quality is especially important when pests are controlled curatively by releases of (often specialist) natural enemies. In that case, pest control mainly depends on the effects of the released natural enemies. In contrast, the quality of the released generalist natural enemies will be less important when they are preventively released, and the offspring of the released natural enemies are responsible for pest control.

Another advantage of the use of generalist predators is that they can establish in crops prior to pest infestation, which makes the system resilient to pest invasion. Thus effective pest management becomes less dependent on the exact timing of releases of natural enemies. In the near future, I expect that biological control systems in greenhouses will increasingly shift from augmentative releases of specialist natural enemies to inoculative releases of generalist predators. For example, whitefly control was mainly based on releases of specialist parasitoids for decades (van Lenteren & Woets, 1988; Avilla et al., 2004). This has changed since the introduction of generalist predatory bugs and predatory mites that also feed on whiteflies. This has been so successful in some crops that most, if not all, biological control is done by means of generalist predators (G. Messelink, personal observations). Thrips control has a long tradition of using generalist predators, and in crops such as sweet pepper, these predators are very effective (Ramakers, 2004). So far, biological control of aphids is mainly based on frequent releases of specialist natural enemies such as parasitoids and predatory midges (Ramakers, 1989; Blümel, 2004), which is expensive and often not successful

(Bloemhard & Ramakers, 2008). Recent experiments showed that inoculative releases of the generalist predator *Macrolophus pygmaeus* can also effectively control aphids in sweet pepper (Messelink et al., 2011). Hence, I expect that future control of aphids and other pests will increasingly be based on generalist predators.

An interesting aspect of using generalist predators is that pest control strongly depends on the diversity of pests in the crop (CHAPTERS 3, 4 and 5). The fact that a mixture of two pests can increase the survival and developmental rate of a generalist predator offers new opportunities to enhance pest control by optimizing the diet for predators. Because many crops do not or hardly provide food for generalist predators, it may be possible to add food that is supplemental to the diet of a certain natural enemy species. Research should furthermore focus on ways to enhance establishment of generalist predators by offering alternative prey in open rearing systems or banker plant systems (Huang et al., 2011), by food sprays (Wade et al., 2008; Messelink et al., 2009), or by selecting plants that provide food or shelter in the crop (Wäckers et al., 2005). Finally, it is desirable that future research focuses on selecting predators that are adapted to certain crop plants and perform well on the pests and food sources present in these crops, rather than selecting natural enemies for any particular pest species.

Summarizing, I conclude that it is important to consider all possible interactions among species in arthropod food webs in order to detect interactions that are potentially detrimental or beneficial for biological control. Detrimental effects can mainly be expected from hyperpredators or hyperparasitoids (CHAPTER 6). In theory, intraguild predation and apparent mutualism can also disrupt biological control. Hence, the results of biological control of a particular pest species may be negatively affected by the presence of other pests or natural enemies. However, I hope to have shown that such negative effects can be outweighed by other, positive effects of generalist predators. Furthermore, the use of a generalist predator for the control of two or more pests can be advantageous for pest control, despite the possibility of apparent mutualism (CHAPTERS 3, 4 and 5). Future research should focus on more complementarity and synergy among natural enemies. There are interesting examples of such interactions, based on predator facilitation (Losey & Denno, 1998), pest stage complementarity (Calvo et al., 2009) or microhabitat complementarity (Onzo et al., 2004).

Nowadays, there are unique possibilities to manipulate communities of natural enemies by choosing from several species that are commercially available (van Lenteren, 2000; Enkegaard & Brødsgaard, 2006). Thus, biodiversity can be created and manipulated to maximise sustainable pest control. At the same time, such systems can be used to study the manipulation of biodiversity and species composition on the dynamics of communities of plant-inhabiting arthropods under relatively controlled conditions. Based on the abundance, diversity and potential risk of pest species, it is possible to adapt the strategies of natural enemy releases. In conclusion, greenhouse experiments that evaluate multiple pest control with diverse assemblages of natural enemies are not only needed to further develop biological control strategies, but also offer excellent opportunities to test and, if necessary, extend theories on multispecies interactions.

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Summary

Plants in agricultural production systems are usually attacked by several species of herbivorous insects and mites. Biological control of these pests can be achieved using specialist and generalist natural enemies. For a long time, biological control was mainly focussed on specialist natural enemies, because they are well adapted to their prey. However, they often cannot persist in a crop when prey are scarce or absent. Repeated introductions are usually needed to control pests, which often involves problems with timing, costs and quality of the natural enemies. In general, generalist predators establish better in crops and can potentially control several pest species. However, they are more involved in various interactions among species than specialists, which can be either detrimental or favourable for pest control.

One of these interactions occurs when generalist predators mediate interactions among pests. These pests can directly influence each other through competition for plant material, but they can also affect each other indirectly by changing the population densities of the generalist natural enemies they share. Theories based on equilibrium dynamics predict that, if a population of a new prey species is added to a system of one predator and one prey species, the equilibrium density of the shared predator will increase and that of the resident prey species will decrease. This is called 'apparent competition', because the dynamics of the two species resemble that of species competing for resources, whereas in fact it is the shared predator that mediates this interaction. In the short term, when dynamical equilibria have not been reached, the predator-mediated indirect interaction between prey may cause the opposite effect; the addition of a population of a second prey species to a predator-prey system leads to satiation of the predator population and consequently lower predation on the resident prey population. In that case, one prey species benefits from the addition of another prey species, which can be classified as 'apparent mutualism'. Such effects may also occur in the long-term in predator-prey systems that show persistent fluctuations. Thus, generalist predators can mediate interactions between pest species that can enhance pest control, but in some cases also can reduce pest control.

Another type of food web complexity occurs when generalist predators consume other natural enemies. This is referred to as 'intraguild predation' when the two species of natural enemies also compete for the same pest species. The predator species that kills and eats natural enemies of another species is called the intraguild predator and the other natural enemy is the intraguild prey. Equilibrium theory on

intraguild predation predicts that when the intraguild prey is a better competitor for the shared pest than the intraguild predator, this will eventually yield less efficient pest control. Predators can also attack other predators with which they do not share a prey (i.e. each predator feeds on a different prey species). I suggest using the term hyperpredation for this kind of interaction, because of its similarity to hyperparasitism (parasitic wasps that parasitize parasitized prey). Hyperpredation can in fact be classified as apparent competition between the alternative prey and the specialist natural enemy. Predation of specialist natural enemies by hyperpredators will release the pest of the specialist natural enemy from control and this effect might become stronger when alternative prey increase the densities of the hyperpredators.

This thesis is on the role of generalist predators in the control of multiple pest species in greenhouse vegetable crops. My first goal was to see whether dynamical patterns predicted by theories of apparent competition, apparent mutualism and intraguild predation could be identified from the dynamics of arthropod communities in greenhouse crops, and second, how interactions in these food webs with generalist predators affected pest control. The pest species that I studied are among the most harmful species in greenhouse crops, namely the greenhouse whitefly, western flower thrips, spider mites and aphids. My research started with the selection and evaluation of different species of generalist predatory mites for the control of thrips in cucumber. Several predatory mite species controlled thrips better than the hitherto commonly used species *Neoseiulus cucumeris*. Strikingly, the most effective predators of thrips, *Typhlodromalus limonicus*, *Amblyseius swirskii* and *Euseius ovalis*, were proven to be capable of controlling whiteflies in other studies. A logical next step was thus to determine how pest control is affected by these predators when both thrips and whitefly were present in a crop. In Chapter 3, I show that both the generalists *A. swirskii* and *E. ovalis* control whiteflies better in the presence of thrips. This appeared a straightforward confirmation of the theory of apparent competition, but something more was going on. The densities of predatory mites were remarkably high when both pests were present, higher than could be explained by the availability of prey. I found that the predatory mite *A. swirskii* developed faster on a mixed diet of whitefly eggs and thrips larvae compared to a diet of thrips only or of whiteflies only. Moreover, there was virtually no mortality during the immature mite stages on a mixed diet, whereas up to 40% of the predators died on a diet of whitefly eggs. Hence, the populations of predators increased faster on a mixture of the two pest species, and the effects of apparent competition seem to be strengthened by this effect of a mixed diet.

In chapter 4, I tested the hypothesis that the interaction between two pests that share a predator may lead to increased pest densities (apparent mutualism) in the short term. This was indeed the case: the control of thrips was reduced by the presence of greenhouse whitefly during the first 3 weeks. However, a strong increase in density of the predatory mites eventually led to better control of thrips with whiteflies present. Satiation effects can occur repeatedly when prey populations show persistent fluctuations, resulting in the repeated occurrence of positive indirect interactions between the prey species. Such fluctuations may occur when young, vulnerable stages that escape from predation due to predator satiation become invulnerable and give rise to a new generation of offspring. This, in turn, can again result in predator satiation, thereby releasing thrips and whiteflies from control. In the experiments described in chapter 4, I mimicked such fluctuations through the release of high numbers of pests at once, which resulted in a high density of a second generation of whiteflies, which indeed resulted in a significant delay of the suppression of thrips populations. Until now, there was little empirical evidence for the occurrence of these effects. With these greenhouse experiments, I show that such effects of fluctuating populations may give rise to a substantial delay in the control of multiple pests with a shared predator population.

In chapter 5, I extended the system of generalist predatory mites, thrips and whiteflies with spider mites, another pest species. First of all, I showed that the predatory mite *A. swirskii* was unable to control spider mites when this was the only pest species present. A laboratory experiment showed that *A. swirskii* was hampered by the web of spider mites, which they produce to protect themselves against various predators. It was therefore surprising that the control of spider mites by this predator was improved in the presence of other pests in a greenhouse trial on cucumber plants. The control of spider mites was better in the presence of thrips than in the presence of greenhouse whiteflies, but the best control occurred in the presence of thrips, whiteflies and spider mites. In this experiment too, the improved pest control was probably caused by the strong population growth of the predatory mites on a mixed diet of thrips and whiteflies. Thus, pest diversity can enhance pest control with generalist predators, even when this pest is a less suitable prey species.

In chapter 6, I show a downside to the use of generalist predatory mites. In greenhouse trials, it became clear that they consume the eggs of an important predator of aphids, the gall midge *Aphidoletes aphidimyza*. This interaction can be classified as hyperpredation, because the mites do not prey on aphids. Hyperpredation of gall midge eggs by the predatory mite *A. swirskii* significantly disrupted the control of aphids in a sweet pepper crop. Hence, this study shows that disruption of aphid control by predatory mites is a realistic scenario and therefore needs to be considered when used in biological control.

In Chapter 7, I compare the effects of two types of generalist predators on aphid control. Specialist natural enemies of aphids (parasitoids and gall midges) were combined with either generalist predatory mites or generalist predatory bugs in a sweet pepper crop that was attacked by aphids and thrips. The predatory mite *N. cucumeris*, a hyperpredator of gall midges, seemed to release aphids from control: densities of aphids were higher in the presence of this predator than when only specialised enemies of aphids were present. The opposite was found for the predatory bug *Orius majusculus*, an intraguild predator of both parasitoids and gall midges; the control of aphids in the presence of this generalist was significantly enhanced compared to the treatment with only specialised aphid enemies. In the laboratory, I showed that these predatory bugs fed on both aphids and thrips when both pests were present. Thrips are likely to contribute to the establishment of the predatory bugs and thereby strengthen the control of aphids, despite the fact that the predatory bugs also feed on the specialist aphid enemies. Hence, this study shows that intraguild predation between natural enemies does not necessarily result in reduced biological control, and it emphasizes the importance of evaluating the effects of generalist predators within food webs of pests and natural enemies.

I conclude that generalist predators can be very valuable for multiple pest control, but that caution is needed because of potential negative effects of generalists on pest control. Biological control in ecosystems with multiple pests and natural enemies therefore requires a systems approach, taking into account the interactions among organisms. Greenhouse experiments that evaluate multiple pest control with diverse assemblages of natural enemies are not only needed to further develop biological control strategies, but also offer excellent opportunities to test ecological theories on multispecies interactions.

Samenvatting

Planten in agrarische teeltsystemen worden vaak belaagd door verschillende soorten plantenetende insecten en mijten. Biologische bestrijding van deze plagen kan met zowel specialistische als generalistische natuurlijke vijanden. Gedurende lange tijd werden voornamelijk specialistische natuurlijke vijanden gebruikt voor biologische bestrijding, omdat deze sterk zijn aangepast aan hun prooi. Een nadeel van specialistische vijanden is echter dat ze zich slecht vestigen in een gewas wanneer hun prooi niet of in lage dichtheden aanwezig is. Hierdoor zijn doorgaans meerdere introducties van de vijanden nodig waardoor de kosten hoog kunnen oplopen. Bovendien is het resultaat sterk afhankelijk van de juiste timing en de kwaliteit van de ingezette natuurlijke vijanden. Generalistische predators vestigen zich over het algemeen beter in gewassen en kunnen meerdere soorten plagen bestrijden. Echter, generalisten zijn, meer dan specialisten, betrokken bij allerlei interacties tussen soorten, en deze interacties kunnen zowel positief als negatief uitpakken voor de plaagbestrijding.

Een van deze interacties treedt op omdat generalisten indirecte interacties tussen plagen veroorzaken. Plagen kunnen elkaar direct beïnvloeden door concurrentie om plantmateriaal, maar ze kunnen ook indirect effect op elkaar hebben doordat ze de dichtheden van een gezamenlijke predator beïnvloeden. Theoretische modellen voorspellen dat de evenwichtsdichtheid van de gezamenlijke predator toeneemt en dat van de prooi afneemt wanneer een nieuwe prooisoort wordt toegevoegd aan een systeem van één predator en één prooisoort. Dit wordt ‘apparent competition’ genoemd (in het Nederlands zoiets als ‘schijnbare concurrentie’), omdat de populatiedynamica van de twee prooien lijkt op de effecten van concurrentie om voedsel, terwijl dit feitelijk wordt veroorzaakt door de gezamenlijke predator. Op de korte termijn, wanneer dichtheden nog niet in evenwicht zijn, kan deze indirecte interactie tussen plagen ook omgekeerde effecten veroorzaken, doordat toevoeging van een prooisoort tot verzadiging leidt van de predators. In dat geval heeft de ene prooisoort dus voordeel bij de toevoeging van een andere prooisoort en is er sprake ‘apparent mutualism’, oftewel ‘schijnbaar mutualisme’. Deze effecten kunnen ook op de lange termijn optreden in predator-prooi-systemen die geen evenwicht bereiken maar waar populaties sterk fluctueren. Generalistische predators kunnen dus interacties tussen plagen veroorzaken die gunstig zijn voor de bestrijding van deze plagen, maar in sommige gevallen kan dit de bestrijding ervan verminderen.

Een andere vorm van complexiteit in voedselwebben met generalistische predators doet zich voor wanneer deze predators andere natuurlijke vijanden aanvallen en doden. Dit wordt aangeduid als ‘intraguild predatie’ wanneer de twee soorten van natuurlijke vijanden ook concurreren om dezelfde prooien. De predator die de andere natuurlijke vijand dood wordt dan de ‘intraguild predator’ genoemd en de andere natuurlijke vijand is dan de ‘intraguild prooi’. Evenwichtstheorie over deze interactie voorspelt dat wanneer de natuurlijke vijand (de intraguild prooi) die wordt aangevallen door de andere natuurlijke vijand (de intraguild predator) superieur is als bestrijder van de gedeelde plaag, dit uiteindelijk zal leiden tot een slechtere bestrijding van die plaag. Predators kunnen ook andere natuurlijke vijanden consumeren waarmee ze geen prooi delen. Deze interactie typeer ik als hyperpredatie, omdat het vergelijkbaar is met hyperparasitisme (sluipwespen die geparasiteerde prooien parasiteren). Hyperpredatie kan in feite worden gezien als apparent competition tussen de alternatieve prooi van de hyperpredator en de gespecialiseerde natuurlijke vijand. Wanneer hyperpredators zich te goed doen aan gespecialiseerde natuurlijke vijanden, zal dat de bestrijding van de prooien van de gespecialiseerde bestrijders verstoren. Dit effect zal sterker zijn wanneer alternatieve prooien de dichtheden van de van de hyperpredators verhogen.

Dit proefschrift gaat over de rol die generalistische predators spelen bij de bestrijding van meerdere plagen in kasteelten van vruchtgroenten. Mijn doel was enerzijds te kijken of de patronen die door de theorieën over apparent competition, apparent mutualism en intraguild predation voorspeld worden ook te herkennen zijn in de populatiedynamica van plagen en predators in kasteelten, en anderzijds, te bepalen in hoeverre dit soort interacties met generalistische predators de biologische bestrijding van plagen beïnvloedt. De plagen die ik heb bestudeerd behoren tot de meest schadelijke soorten in de glastuinbouw, namelijk de kaswittevlieg, Californische trips, spintmijt en bladluis. Het onderzoek startte met de selectie en vergelijking van verschillende soorten generalistische roofmijten voor de bestrijding van trips in komkommer. Verschillende roofmijtsoorten gaven een effectievere bestrijding van trips dan de tot dan toe veelgebruikte soort *Neoseiulus cucumeris*. Opvallend was dat de meest effectieve roofmijten *Typhlodromalus limonicus, Amblyseius swirskii* en *Euseius ovalis,* in andere studies ook bestrijders van wittevlieg bleken te zijn. Een logisch gevolg was te bepalen hoe de plaagbestrijding met deze predators verloopt wanneer zowel trips als wittevlieg in hetzelfde gewas aanwezig zijn.

In Hoofdstuk 3 laat ik zien dat de generalisten *A. swirskii* en *E. ovalis* beiden kaswittevlieg beter bestrijden wanneer ook trips aanwezig is. Dit leek een simpele bevestiging van de theorie van apparent competition te zijn, maar er was meer aan de hand. De dichtheden van roofmijten waren opvallend hoog bij de aanwezigheid van beide plagen. Ik vond dat de roofmijt *A. swirskii* zich sneller ontwikkelde op een gemengd dieet van tripslarven en wittevliegeieren ten opzichte van een dieet van alleen trips of wittevlieg. Bovendien was er bij het gemengde dieet nagenoeg geen sterfte van de onvolwassen roofmijtstadia, terwijl bij het dieet van alleen wittevliegeieren tot 40% van de roofmijten stierf. De effecten van apparent competition lijken dus versterkt te worden door het gunstige effect van het gemengde prooidieet op de predatorpopulatie.

In hoofdstuk 4 toets ik de hypothese dat de interactie tussen twee plagen die een predator delen op de korte termijn kan leiden tot verhoogde plaagdichtheden (apparent mutualism). Dit bleek inderdaad het geval te zijn: bestrijding van trips werd in de eerste 3 weken vertraagd door de aanwezigheid van kaswittevlieg, maar door de sterke numerieke respons van de roofmijten leidde dit uiteindelijk tot een betere tripsbestrijding in komkommer. Verzadigingseffecten kunnen zich herhaaldelijk voordoen wanneer prooipopulaties aanhoudend schommelen, wat resulteert in het frequent optreden van positieve indirecte interacties tussen de prooisoorten. Die schommelingen kunnen optreden doordat jonge kwetsbare stadia door verzadiging van de predators ontsnappen aan predatie waardoor ze zich ontwikkelen tot onkwetsbare stadia die weer een nieuwe generatie van nakomelingen produceren. Dit kan op zijn beurt weer resulteren in verzadiging van de predators waardoor trips en wittevlieg opnieuw ontsnappen aan predatie. In de experimenten beschreven in hoofdstuk 4 heb ik dergelijke schommelingen geprobeerd experimenteel na te bootsen door het gelijktijdig loslaten van veel plaagindividuen. Dit resulteerde in een hoge dichtheid van een tweede generatie van wittevlieg, hetgeen inderdaad resulteerde in een aanzienlijke vertraging van de onderdrukking van de tripspopulatie. Tot nu toe was er weinig empirisch bewijs voor het optreden van deze effecten. Met deze kasexperimenten laat ik zien dat dergelijk effecten van schommelende populaties kunnen leiden tot een aanzienlijke vertraging van de plaagbestrijding.

In hoofdstuk 5 heb ik het systeem van een generalistische roofmijt, trips en wittevlieg verder uitgebreid met spint. Als modelpredator heb ik opnieuw de roofmijt *A. swirskii* gebruikt. Allereerst laat ik zien dat deze roofmijt niet in staat is om spint op komkommer te bestrijden wanneer dit de enige plaag is. In het laboratorium bleek dat de spintpredatie door deze rover belemmerd wordt door het web waarmee spintmijten zich beschermen tegen allerlei predators. Het verrassende was dat in kasproeven op komkommer de bestrijding van spint met *A. swirskii* verbeterd werd door de aanwezigheid van andere plagen. De bestrijding van spint verliep beter in aanwezigheid van trips dan in aanwezigheid van kaswittevlieg, maar de beste bestrijding van spint vond plaats op planten met zowel trips als wittevlieg. Ook in dit experiment kon verbeterde plaagbestrijding waarschijnlijk verklaard worden door de sterke populatiegroei van roofmijten op een gemengd dieet van trips en wittevlieg. Plaagdiversiteit kan dus de plaagbestrijding met generalistische predators verbeteren, zelfs van plagen die in eerste instantie ongeschikt lijken als prooi.

In hoofdstuk 6 laat ik zien dat er ook een keerzijde zit aan het gebruik van generalistische roofmijten. In kasproeven bleek dat ze zich voeden met de eieren van een belangrijke bestrijder van bladluis: de galmug *Aphidoletes aphidimyza*. De roofmijten zijn geen predators van bladluis en kunnen daardoor bestempeld worden als hyperpredators. Hyperpredatie van galmugeieren door de roofmijt *A. swirskii* resulteerde in een slechtere bestrijding van bladluis in paprika. Bij inzet van generalistische roofmijten tegen trips, wittevlieg en spint, moet er dus rekening worden gehouden met mogelijk negatieve effecten op de bestrijding van bladluis met galmuggen.

In Hoofdstuk 7 vergelijk ik de effecten van roofmijten met roofwantsen op bladluis in een paprikateelt met trips en met specialistische bladluisbestrijders bestaande uit sluipwespen en galmuggen. De roofmijt *N. cucumeris*, een hyperpredator van galmug, leek de bestrijding van bladluis iets te verslechteren ten opzichte van een gewas met alleen bladluisbestrijders. De roofwants *Orius majusculus*, een intraguild predator van zowel sluipwespen als galmuggen, bleek de bestrijding van bladluis juist aanzienlijk te verbeteren ten opzichte van de behandeling met alleen bladluisbestrijders. In het laboratorium laat ik zien dat deze predator zich met zowel trips als bladluis voedt wanneer beide plagen aanwezig zijn. De trips kan daardoor bijdrage aan de vestiging van de roofwantsen en daarmee de bestrijding van bladluis verbeteren, ondanks het feit dat de roofwantsen zich ook voeden met de specialistische bestrijders van bladluis. Deze studie geeft dus aan dat intraguild predatie tussen natuurlijke vijanden niet per definitie tot een slechtere bestrijding van plagen hoeft te leiden. Dit benadrukt het belang om effecten van generalistische predators op plagen te testen in de juiste context van plagen en bestrijders.

Mijn conclusie is dat generalistische predators zeer waardevol kunnen zijn bij de bestrijding van meerder plagen gelijktijdig, maar dat het belangrijk is om oog te hebben voor mogelijke negatieve effecten. Biologische bestrijding in ecosystemen met meerder plagen en bestrijders vraagt dus om een systeembenadering waarbij rekening wordt gehouden met de onderlinge interacties tussen organismen. Kasexperimenten waarbij de bestrijding van meerder plagen met verschillende groepen van natuurlijke vijanden worden geëvalueerd zijn niet alleen van belang voor het verder ontwikkelen van biologische bestrijdingssystemen, maar ook voor het testen van ecologische theorieën over interacties tussen organismen in voedselwebben.

Curriculum vitae

Gerben Messelink was born on March 2, 1973 in Doornspijk ('t Harde), a small village on the forest-rich 'Veluwe' in The Netherlands. After high school (VWO, Lambert Franckens College), he studied plant breeding and crop protection at the Wageningen Agricultural University with a specialization in ecological crop protection (phytopathology and entomology). During this study, he stayed 6 months at the Malborough Wine Research Centre in New Zealand. After his graduation in 1997, he began his first job for the company Relab den Haan, dealing with plant health diagnostic services for the greenhouse industry. With two years of experience in identifying pests and pathogens, he started to work as a phytopathologist at the Research station for Floriculture and Glasshouse Vegetables in Naaldwijk. In 2000, he switched to a new job within the same institute as a scientific researcher entomology. This institute was privatised in 2001 and changed her name in Applied Plant Research and eventually in Wageningen UR Greenhouse Horticulture. Since the switch to entomology, he worked on many applied projects on pest control, such as the cabbage root fly in radish, *Duponchelia fovealis* in potted plants, bulb scale mite in amaryllis, thrips in chrysanthemum, thrips, spider mite and whitefly in cucumber and caterpillars and aphids in sweet pepper. His work on predatory mites in cucumber was in close collaboration with the University of Amsterdam, which resulted in a number of joined publications and this thesis. He is active within the international organization for Biological Control (IOBC) as council member of the WPRS section.

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Dankwoord

Dit proefschrift was niet tot stand gekomen zonder de hulp en inzet van veel verschillende mensen. Allereerst Arne Janssen, mijn begeleider gedurende dit traject. Het moet ergens in 2007 zijn geweest toen je mij vroeg om naast mijn werk te gaan promoveren bij de UvA. Een vraag die mij enigszins overrompelde, maar wat uiteindelijk uitpakte in een mooie en langdurige samenwerking. Arne, ik wil je dan ook hartelijk bedanken voor dit aanbod dat je me destijds gaf. Onze interactie vond en vindt vooral plaats in het schrijfproces van publicaties. Dit heb ik als zeer prettig, leerzaam en stimulerend ervaren en je enthousiasme werkte aanstekelijk! Het is ongelofelijk hoe snel en behulpzaam jij altijd reageert op emails. Enorm bedankt voor al je inbreng en betrokkenheid! Maus Sabelis, mijn promotor, bedankt voor de vele stimulerende discussies en volstrekt originele gedachtenspinsels. Het was vooral leuk je intensief mee te maken op de IOBC congressen in Florence, Sint Michielgestel en tijdens de reis met Koppert naar Almeria. Ik had het bijzondere geluk dat dit traject gelijk op liep met het promotieonderzoek van Roos van Maanen. Roos, super dat we konden samenwerken! We vulden elkaar mooi aan en het was altijd erg gezellig!

Uiteraard was veel van dit werk niet mogelijk geweest zonder een intensieve samenwerking met mijn collega's van Wageningen UR. Ik heb bijzonder veel te danken aan Pierre Ramakers die mij wegwijs heeft gemaakt in de wereld van de biologische plaagbestrijding. Pierre, dank voor het vertrouwen dat je me hebt gegeven om jouw werk deels voort te zetten. De expertise die jij hebt opgebouwd binnen ons instituut is een ware zegen geweest. En dan Renata, Chantal en Sebastiaan, met jullie heb ik intens samengewerkt in het onderzoek van dit proefschrift. Het was, en is, geweldig om met jullie samen te werken door jullie betrokkenheid, gezelligheid en meedenken in alle proeven. Ik ben jullie enorm dankbaar! En Sebastiaan, nog altijd jammer dat je onze groep hebt verlaten voor het onderwijs. Je was een supercollega! Bij veel experimenten komt intensief telwerk om de hoek kijken. Laxmi, Wim, Eric en Marc, bedankt voor jullie toewijding, zorgvuldigheid en paraatheid. Verder is het een geweldige luxe om te kunnen 'sparren' met collega entomologen. Anton, Amir, Ellen, Juliette en Marieke, bedankt voor jullie gezelligheid en samenwerking. Het gezamenlijk organiseren van het IOBC congres in Sint Michielsgestel vond ik persoonlijk toch wel een hoogtepunt. Mooi te zien hoeveel energie er vrij komt bij zo'n operatie. Rob, ik wil jou als 'mijn baas' bedanken voor het vertrouwen dat je mij de afgelopen jaren hebt gegeven in het onderzoek en de mogelijkheden die je schept om me verder te ontwikkelen. Verder de andere collega's met wie ik de afgelopen jaren heb samengewerkt: Ada, Aleid, André, Bertin, Chris, Daniel, Dirk-Jan, Geo, Ineke, Iris, Jan, Jantineke, Jos, Marrie, Marie-Anne, Marta, Pim, Roel, Rozemarijn, Wim: bedankt! En ook de mannen van de tuin, met name Kees, Johan en Fred: dank voor jullie inzet bij kasproeven. Juan Cortez, thanks for your contribution to chapter 6. It was great to work with you!

Veel van mijn onderzoek in komkommer is op de voet gevolgd door de telers Maarten Post en Leen verhaar. Dank voor jullie steun aan het onderzoek! De eerste praktijkproeven met *A. swirskii* zijn in samenwerking met Steven Voet gedaan. Dankzij de overtuigende resultaten daarvan konden we doorgaan met het onderzoek. Bedankt Steven voor je inzet! Mijn werk in paprika werd een stuk dynamischer door de nauwe betrokkenheid van biologische paprikatelers die de problemen met bladluis aan den lijve ondervinden. Vooral de telers van mijn begeleidingscommissie bij dit onderzoek ben ik zeer dankbaar: Frank de Koning, Tonnie Vink en Jac Verbeek. De discussies met jullie zijn altijd zeer stimulerend en constructief.

Het R&D team van Koppert wil ik zeer bedanken voor de samenwerking. Karel, Hans, Jeroen, Yvonne: het is geweldig leuk werken met jullie! Het was mooi om betrokken te zijn bij de opmars van *A. swirskii* in de praktijk. Het maken van de speciale swirskii-website met Markus en Rogier was daar een leuk onderdeel van. De dynamiek was ongekend.

Jan Bruin wil ik bedanken voor de vormgeving van dit boekje.

Tot slot op de achtergrond natuurlijk Jolanda, mijn lieve sterke vrouw en moeder van onze 4 prachtige kinderen. Dank voor al je ondersteuning de afgelopen jaren. Job, Olaf, Bento en Rhodé, jullie relativeren alles wat in het werk zo belangrijk lijkt.

Gerben