What lies beneath?

Linking litter and canopy food webs to protect ornamental crops

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General introduction

This thesis is on biological control, which is the use of live organisms to keep agricultural pests, diseases, and weeds at low levels (DeBach 1974). Since ancient times, humans have utilized natural enemies to control pests. Thousands of years ago, ants and spiders were used in China to protect tree crops from herbivore pests. Through history, there have been many successful programs of biological control worldwide (Van Driesche & Bellows 1996). Only after World War II, the use of chemical pesticides replaced natural and biological control (Bale et al. 2008). Thanks to the pressure of consumers for pesticide-free agricultural products, biological control has again become a key component for pest control (Calvo et al. 2015). Moreover, growers have actively chosen to use biological control because of the advent of pests resistant to chemical pesticides (Bolckmans 1999). Nowadays, biological control is a viable alternative to chemical pest control.

Currently, there is a wide range of biological control agents commercially available, such as parasitoids, entomopathogens and predators. Parasitoids are wasps and flies that deposit their eggs in or on their host. In the case of biological control, these hosts are insect pests. The developing parasitoid larva feeds on the haemolymph of the host, affecting the host’s development and finally causing its death (Godfray 1994). Entomopathogens used in biological control are viruses, fungi, bacteria, and protozoa that cause diseases in insects and mites (Federici 1999). These pathogens infect arthropod pests, eventually killing them (Federici 1999). In this thesis I focus on arthropod natural enemies. Arthropod predators living on crop plants can be either specialist, attacking one or a few closely related pest species, or generalist, attacking a wider range of pests. In the past, biological pest control has focused on the use of one or a few specialist natural enemies for each pest species (Huffaker & Messenger 1976; Hokkanen & Pimentel 1984; van Lenteren & Woets 1988; Hoy 1994) because the use of generalist predators was judged to be more risky as they could also target non-pest species (van Lenteren et al. 2003). Moreover, they were considered inferior enemies to parasitoids because their dynamics would not be synchronized with the pest, and because they usually do not have a high potential for increase (Murdoch et al. 1985). Nevertheless, generalist predators are widely used in biological control nowadays (Symondson et al. 2002). These predators have the advantage of being able to feed on food sources different than the target pest. Thus, they are able to establish in the crop before pest invasions (Symondson et al. 2002). The introduction of different natural enemies (either gener-
alists, specialists, or both) results in artificial communities in the crops, and the members of those communities can interact in complex ways. Not only do natural enemies interact with the target pest, but interactions also occur among different pest species and among natural enemy species (Helle & Sabelis 1985; Minks & Harrewijn 1988; Sabelis 1992), and these interactions can affect biological control (Sih et al. 1985; Janssen et al. 1998; Prasad & Snyder 2004; Evans 2008). A lot of attention has been given to the effects of interactions among various species of natural enemies. Compared to the effect of each enemy species separately, the effect of such interactions can be negative, neutral or positive for biological control (Rosenheim et al. 1995; Losey & Denno 1998; Rosenheim 1998; Colfer & Rosenheim 2001; Snyder & Ives 2001; Venzon et al. 2001; Cardinale et al. 2003; Snyder & Ives 2003; Finke & Denno 2004; Cakmak et al. 2009).

Interactions among natural enemies

When multiple species of arthropod natural enemies are present in a crop, there can be negative effects on biological control. Especially generalist predators can interfere with other natural enemies not just through competition for prey or food, but also through hyperpredation or intraguild predation (Rosenheim et al. 1995; Rosenheim 1998; Snyder & Wise 2002). Intraguild predation takes place when two natural enemies that attack the same prey are also involved in predation (Polis & Holt 1992; Ferguson & Stiling 1996). Intraguild predation is common in food webs in crops and its effects can be strong, resulting in low survival of one or both natural enemies, disrupting biological control (Rosenheim et al. 1995; Rosenheim & Harmon 2006; Janssen et al. 2007; Vance-Chalcraft et al. 2007; but see Janssen et al. 2006).

The use of multiple predators can also result in positive effects on biological control (Losey & Denno 1998). A predator with a certain foraging behaviour can cause behavioural changes in a particular prey, making it more available to other predator species (Sih et al. 1998). For example, Losey & Denno (1998) show that better biological control of aphids is achieved with two predators instead of either one separately. The mechanism for this positive effect is that a canopy-dwelling predator induced ‘dropping’ behaviour in the aphids, which makes them available to a predator on the soil. Yet other studies show that the effect of the presence of multiple predators on pest control can be neutral, meaning that the multiple predator effects on the pest population correspond to the sum of the single-species effects (Gillespie & Quiring 1990; Sokol-Hessner & Schmitz 2002; Wiethoff et al. 2004), which would still result in better biological control.

Summarizing, the indirect interactions among arthropods in food webs with generalist arthropod predators are complex and need more study. To make adequate use of generalist predators for biological control, it is crucial to study their interactions with all pests and predators present in a crop. In this thesis, I studied the combined effect of multiple predator species on the control of one pest species.

Interactions among pest organisms

Herbivorous prey might compete among each other for plant resources, but this only occurs at high pest densities, which is undesirable in agriculture because it would certainly result in exceeding the economic damage threshold. For this reason, I will not further discuss resource competition among herbivores in this thesis. Herbivores can also affect each other indirectly by inducing plant defence responses: the attack of an herbivore triggers a plant defensive response that does not only affect the inducing herbivore, but also other herbivore species on the same plant (Karban & Carey 1984). Plants have direct defences against herbivores, for example, they might produce toxins or anti-feeding compounds, resulting in reduced survival, fecundity or developmental rate of the herbivore (Kessler & Baldwin 2002). The structure and composition of herbivore communities are often shaped by plant-mediated interactions due to induction of plant defences (Karban & Baldwin 1997; Kessler & Hallischke 2007). For example, resistance against leaf miners increases when tomato and cucumber plants are previously attacked by whiteflies (Inbar et al. 1999; Zhang et al. 2005). In contrast, susceptibility to aphids increases when tomato plants were previously attacked by whiteflies (Nombela et al. 2009). Plant defences can also be down-regulated, the spider mite Tetranychus evansi suppresses plant defences (Sarmiento et al. 2011a), which enhances the mite’s performance, as well as that of the closely related spider mite Tetranychus urticae (Sarmiento et al. 2011b).

Plant defences can also be indirect by affecting the action of the natural enemies of the herbivores (see Sabelis et al. 1999 for a review). They can do this by providing food or shelter to the natural enemies, thus arresting the natural enemies on the plant, and increasing their survival or reproduction. Plants are also known to produce specific blends of volatiles upon attack by herbivores, and these volatiles are attractive to natural enemies (Turlings et al. 1990; Dicke & Sabelis 1988) and herbivores can thus also affect each other through the induction of plant volatiles. For example, Shioji et al. (2002) show lower parasitism of P. xylostella on cabbage plants co-infested with another herbivore (Pieris rapae) than on plants infested only with P. xylostella, because the parasitoids of P. xylostella are not attracted to the volatile blends produced by plants that are attacked by both herbivores. The opposite effect is recorded on cabbage plants with the parasitic wasp of P. rapae, which attacked more hosts on plants attacked by both herbivores (Shioji et al. 2002).

Herbivores can also affect each other through structural changes of the environment. For example, several species of spider mites cover the leaf area on which they feed with a dense web, which is thought to serve as protection against predators...
(Sabelis & Bakker 1992), but this web can also serve as refuge for other herbivores (Pallini et al. 1998; Magalhães et al. 2007), but may exclude yet other herbivores (Sarmento et al. 2011b). Concluding, interactions among herbivores might affect biological pest control in crops.

Different prey species can also affect each other indirectly through a population of generalist predators and this is one of the main themes of this thesis. For example, when a generalist predator feeds on a target pest and on an alternative prey, the alternative prey can indirectly affect the densities of the pest (Holt 1977). The presence or addition of alternative food or prey to a population of generalist predators can result in positive effects on the pest species (apparent mutualism; Holt 1977). For example, the predator can direct its attacks to the alternative food or prey and thus become satiated, resulting in fewer attacks of the pest (apparent mutualism; Holt 1977), negatively affecting biological control. However, after one or a few generations, the increased availability of food will boost the densities of the predators, resulting in increased predation of both the target pest and the supplied food (apparent competition; Holt 1977), thus biological control is increased. When populations of natural enemies and pests show fluctuations, periods of positive effects of the two prey on each other will alternate with periods of negative effects (Abrams & Matsuda 1996). Whereas apparent mutualism is undesired when supplying alternative food, apparent competition is beneficial for biological control (van Rijn et al. 2002). The occurrence of apparent competition in biological control systems has been widely studied (Collyer 1964; Karban et al. 1994; Hougen-Eitzman & Karban 1995; Müller & Godfray 1997; Liu et al. 2006; Messelink et al. 2008). For example, Karban et al. (1994) shows that releasing an economically unimportant herbivore together with predatory mites increases the biological control of Pacific spider mites, a pest of grapevines. Hence, apparent competition between herbivores and between a pest population and alternative food can improve biological control. In this thesis, I present several examples of this.

An important aspect that has not been incorporated in the theory of apparent competition and apparent mutualism is the effect of a mixed predator diet. Depending on the quality of the prey or food source, the performance of generalist predators can be affected by feeding on a mixed diet (Uetz et al. 1992; Toft & Wise 1999; Oelbermann & Scheu 2002; Messelink et al. 2008; Marques et al. 2015). For example, the fecundity of the predatory mite *Homeoprornematus anconai* (Baker) is significantly increased when it feeds on its prey plus pollen (Hessein & Perring 1988). Messelink et al. (2008) found a shorter immature development of the predatory mite *Amblysieus swirskii* (Athias-Henriot) when feeding on two prey (thrips and whiteflies) compared to predators that feed on either of the two prey separately. Other studies show that a mixed diet can have positive or negative effects on performance of generalist predators, depending on the combinations (Toft & Wise 1999; Oelbermann & Scheu 2002).

A meta-analysis performed by Lefcheck et al. (2013) shows that animals that feed on mixed diets have higher fitness than animals feeding on single diets.

A question that arises is why a mixed diet affects performance of generalist predators. There are two possible reasons. Firstly, different prey may provide the predators with different essential nutrients. With certain mixed diets, predators can acquire the nutrients needed to reproduce more or develop faster compared with a single food or another mixed diet (Uetz et al. 1992; Lefcheck et al. 2013). Another reason is that, by mixing different prey or foods, predators can avoid ingesting harmful quantities of poisonous or growth-reducing substances from each food or prey separately (Coll & Guerson 2002; Lefcheck et al. 2013). In order to design a food web that enhances pest control, it is crucial to assess the effects of mixed diets on the performance of predators. In this thesis, I studied the effects of mixed diets on the performance of generalist predatory mites used for biological control.

**Pollen as alternative food**

Many species of plants produce pollen that are edible for arthropods, including predators and parasitoids. Thus, plants invoke a kind of apparent competition between the pollen and the pests attacked by these natural enemies. For some natural enemies, pollen is a highly nutritious food. It contains proteins, lipids, carbohydrates, and minerals (Lundgren 2009). The presence of edible pollen can result in the persistence of populations of arthropod predators in the absence of their prey. Currently, the pollen of some plant species, such as *T. palustris* spp., are being used in Europe as an alternative food for generalist predators in greenhouse crops (Vangansbeke et al. 2014; Pijnakker et al. 2016a). A risk of adding pollen to crops is that insect pests such as thrips can benefit from it (Chitturi et al. 2006; Leman & Messelink 2015; Vangansbeke et al. 2016). However, studies have shown that pollen can help boosting the populations of generalist predators, increasing biological control of thrips and herbivorous mites (van Rijn et al. 1999; Nomikou et al. 2002; Duarte et al. 2015). For example, van Rijn et al. (2002) added pollen as alternative food for phytoseiid mites and found increased control of thrips on cucumber plants compared with treatments in which pollen was not added.

Contrasting results have also been found. Delisle et al. (2015) show that by adding pollen as alternative food for predatory mites, thrips cause more damage than when pollen has not been added. However, the duration of the experiment may have been too short to observe a positive effect of adding pollen for predators on thrips control (i.e. the authors observed short-term apparent mutualism, but no longer-term apparent competition between the pollen and the pest). Although the addition of pollen and other alternative foods to the above-ground parts of a crop often does seem to result in better pest control, the disadvantage is that these alternative foods may contam-
inate the marketable parts of the crop. This is especially the case for ornamentals. I therefore investigate the possibilities of adding alternative food for predators to the litter instead of to the plant canopy. This requires investigating the connections between food webs occurring in the litter and the above-ground plant parts.

**Links between above-ground and litter food webs**

One clear distinction between the food web occurring above-ground and that occurring in the litter is the presence of detritivores. These organisms usually do not cause damage to the plants, but have an important role in recycling nutrients and minerals from the litter (Seastedt 1984). Coincidentally, several detritivore species are used as food for mass production of natural enemies, hence, there is potential for using the litter food web as a source of alternative food for predators. Although the two food webs were often studied in isolation, it is now clear that the above-ground and below-ground communities associated with plants are strongly interlinked (Scheu 2001; van der Putten et al. 2001, 2009; Miyashita et al. 2003; Moore et al. 2003; Wardle et al. 2004). For example, some generalist predators can switch from feeding on detritivores below-ground to herbivores in the canopy, thus forming a direct link between the two (Scheu 2001). There are a few examples of biological control systems in which the link of above-ground and below-ground food webs is investigated (Settle et al. 1996; Halaj & Wise 2002; Birkhofer et al. 2008; Von Berg et al. 2009). Settle et al. (1996) increase organic matter in rice fields, thus enhancing populations of decomposers and plankton feeders, resulting in a significant increase in the abundance of spiders and insect predators and a decrease of above-ground herbivores. Contrasting results are reported by Birkhofer et al. (2008), who show that an increase in decomposer prey densities in wheat fields results in an increase of pest populations above-ground 1 month later. The authors conclude that increasing the densities of decomposers results in shifts in the foraging of beetles and spiders, predators that typically have long generation times, reducing predation of the pest herbivores (reminiscent of apparent mutualism; Holt 1977; Abrams & Matsuda 1996). Another study shows that generalist predators feed on organisms from the above-ground and below-ground food webs, having an effect on above-ground pest densities (Von Berg et al. 2009). Although most of these studies show that the below-ground food web and the herbivore food web on the above-ground plant parts are connected through generalist predators, many of them did not study population dynamics of predators and pests and the impact on plant damage and thus on biological control. In this thesis, I therefore investigate interactions among and between food webs on above-ground plant parts and the litter and how these interactions affect biological pest control in a rose crop (Box 1). Food web interactions in such a system can be very complex (Figure 0.1), and the effect of these interactions are likely to affect biological control. Studying these interactions is important for two reasons: first, it can help to design food webs that allow growers to improve biological control programs, and second, it can help to test ecological theories. In this thesis, I specifically study the effects of apparent competition and apparent mutualism and the interactions between above-ground and below-ground food webs on pest densities.

**Figure 0.1.** A food web associated with roses in a greenhouse. (1) Plant resources: (1a) rose plants; (1b) pollen, which serves as food for some pests and predators. (2) Detritivores in the litter can be used as alternative prey for predators. (3) Pest species: (3a) spider mite eggs; (3b) thrips, which cause damage on above-ground plant parts and spend part of their life cycle as pre-pupae and pupae in the litter or soil; (3c) whitefly eggs. Notice that I have depicted eggs of spider mites and whiteflies, which do not feed on plants, but the other stages of these pest species do, hence the solid arrows. (4) Generalist predators, which connect above-ground and litter or soil food webs by feeding on pests, and by feeding on herbivores and alternative prey in the litter or soil: (4a) Amblyseius swirskii; (4b) Balaustium leanderi; (4c) Cosmolaelaps n. sp.; (4d) Macrocheles robustulus.
Box 1: Cut-rose production

Biological control strategies commonly used for vegetable crops do not always work in ornamentals (Parrella et al. 1999). This is because damage thresholds are much lower for ornamentals than for vegetable crops: for example, the presence of thrips or thrips damage is unacceptable for cut-flower commercialization. Thus, growers of ornamental crops often spray chemical pesticides and fungicides regularly and year-round (Parrella et al. 1999). In order to use biological control as a feasible alternative to chemical pesticides, natural enemies must therefore be efficient in maintaining ornamentals free from pests.

Cut-rose production is important worldwide, it provides thousands of jobs in many countries (Parrella et al. 1999). In some countries, like The Netherlands, roses are grown using a technique developed in Japan, known as arching (Ohkawa & Suematsu 1999). Growers buy budded stock plants from propagator companies. The substrate used is rockwool; fertilizers dissolved in water are provided through the irrigation system. Under these conditions, the rose plants can last for around 10 years (Parrella et al. 1999). During this time, rose plants produce a large amount of litter (Figure 0.2a). For practical reasons, this is disposed below the gutters, a special kind of benches where the plants on the rockwool are growing (Figure 0.2b). From each rose plant, a few stems are selected to produce marketable roses, the other stems are bent, but remain attached to the plant (Ohkawa & Suematsu 1999). The bent stems connect the litter layer to the foliage of the plants (Figure 0.2a). In rose crops, the food web within the litter or soil layer has not been studied before, nor the interactions among the organisms belonging to this food web, and with the food web on the above-ground plant parts.

Figure 0.2. Characteristics of commercial rose crops in The Netherlands. (a) Litter in a commercial rose greenhouse. (b) Diagram of a commercial rose production system.

Synopsis

The main research question of this thesis is how interactions between above-ground and below-ground food webs affect biological control. In Chapter 1, I investigated the effect of a mixed diet of above-ground plant pests on life history traits of a generalist below-ground predator, Balaustium leanderi, which also forages on the above-ground plant parts (Box 2). Females of B. leanderi lay one batch of eggs in 1 to 2 days at the end of their lives, exhibiting big-bang reproduction and their life cycle last c. 2 months. I recorded life history traits such as reproductive performance, survivorship and development when fed on mixed diets of three pest species that inhabit above-ground plant parts (Box 3).

In Chapter 2, I further investigated generalist predators that link above-ground and litter food webs. The canopy-dwelling predator A. swirskii mainly inhabits the above-ground plant parts but makes excursions to the litter layer (Box 2). I tested whether the addition of astigmatic mites (Box 4) as alternative prey in the litter would boost predator densities, resulting in better pest control (apparent mutualism or apparent competition, Holt 1977). I addressed the following questions. 1) Do predators move from the canopy to the litter to feed on an alternative prey? 2) Do predators still control the pest even if the quality of the alternative prey in the litter is higher than the pest? 3) Do pest densities and plant damage decrease when providing alternative prey in the litter?

Subsequently, I investigated the effect of litter-inhabiting predators (Box 2) present in commercial rose production greenhouses on thrips control (Chapter 3). Many pest species, including thrips, spend part of their life cycle in the litter or soil (Wahab 2010), so litter-inhabiting predators may contribute to thrips control. I specifically tested whether supplying food for these predators helps to increase biological control of thrips. Growers commonly release canopy-dwelling predators to control pests (Branigan 1915; DeBach 1974; Gerson & Weintraub 2007), and these may interact with litter-inhabiting predators, which may affect pest control. Thus, in Chapter 4 I investigated the combination of species of canopy-dwelling and litter-inhabiting predator species. Combining natural enemies sometimes results in increased biological control compared with single releases of either of the natural enemy species separately (Losey & Denno 1998, 1999; Sih et al. 1998; Casula et al. 2006). For this reason, and because pests such as thrips inhabit above-ground plant parts and the litter or soil, attempts have been made to increase thrips control by combining canopy-dwelling predators and soil-inhabiting predators (Wiethoff et al. 2004; Thoeming & Poehling 2008; Manners et al. 2013; Pozzebon et al. 2015). The novelty of my study is that I investigated whether biological control can be enhanced by supplying different alternative food sources for canopy-dwelling and litter-inhabiting predators (pollen or astigmatic mites).
One of the most successful groups of arthropods used to control pests in greenhouses are predatory mites (Gerson & Weintraub 2007). After being released, they can rapidly establish in the crops; they oviposit on different parts of the plants, including the lower stratum of the canopy. This is important because not all predatory mites are removed when harvesting flowers. The main predatory mite species studied in this thesis are *Balaustium leanderi* (Haitlinger) (Actinotrichida: Erythraeidae), *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae), *Cosmolaelaps n. sp.* (Acari: Laelapidae) and *Macrocheles robustulus* (Berryse) (Acari: Macrochelidae). These predatory mites are chosen because they either occur in rose greenhouses or are released commercially in rose crops.

*Balaustium leanderi* (Figure 0.3a) is a generalist predator which is naturally found in Mexico and Colombia (Getiva & Acosta 2004; Fuentes et al. 2014). It inhabits the soil or litter layer in roses and other crops, where the applications of chemical pesticides are not frequent (Muñoz-Cárdenas et al. 2015). Some species of the genus *Balaustium* have been studied in regard to their potential use in biological control of agricultural pests (Cadogan & Laing 1977; Makol et al. 2012; Chapter 1). Due to difficulties rearing these predatory mites, biological control studies are scarce. However, studying the potential for pest control of these predatory mites is important because they are well adapted to crop conditions and they are found attacking different pest species in America and Europe (Muñoz-Cárdenas et al. 2015).

*Amblyseius swirskii* (Figure 0.3b) is a predator from the Mediterranean region (Swirskii et al. 1967; Nomikou et al. 2001). This predator is widely used in Europe and North America to control pests in vegetable and ornamental crops like roses (Pijnakker & Ramakers 2008; Buitenhuis et al. 2015; Calvo et al. 2015). In vegetable crops, *A. swirskii* successfully controls whiteflies and thrips (Nomikou et al. 2002; Messelink et al. 2008; Nomikou et al. 2010), but it can also control herbivorous mites (van Maanen et al. 2010) and feeds on other alternative food sources and pest species (Hoogerbrugge et al. 2008; Calvo et al. 2015; Janssen & Sabelis 2015; Vangansbeke et al. 2016). This predator inhabits the plant canopy and moves to the litter or soil to disperse (Buitenhuis et al. 2010) or to feed on alternative prey (Chapter 2).

*Cosmolaelaps n. sp.* (Figure 0.3c) inhabits the litter layer in rose greenhouses in The Netherlands (Chapter 3). Individuals of this genus feed on nematodes, astigmatic mites, and thrips (de Moraes et al. 2015). A species of this genus found in Brazil, *Cosmolaelaps jaboticabalensis*, is considered to have potential to control thrips (Furtado & de Moraes 2015).

*Macrocheles robustulus* is a generalist predator that also inhabits the soil in greenhouses in The Netherlands. It feeds on astigmatic mites and it is commercially released in greenhouses to control insect pests like thrips (Messelink & van Holstein-Saj 2008).

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**Box 3: Target pests**

The following pest species were studied in my thesis because they are cosmopolitan and represent some of the most important pest species in crops in the field and in greenhouses: the western flower thrips *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) (Figure 0.4a); the two-spotted spider mite *Tetranychus urticae Koch* (Acari: Tetranychidae) (Figure 0.4b) and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) (Figure 0.4b).

The two-spotted spider mite is known to attack over 1100 plant species (Migeon & Dorkeld 2016). It causes leaf decoloration by feeding on chlorophyll. Spider mites punc-
piercing epidermal and parenchymal cells, and sucking out their contents. They also transmit viruses (Brødsøgaard & Albajes 1999). Damaged plant parts develop characteristic silvery spots or scars. In ornamentals, low densities of thrips can cause aesthetic damage to flowers, the marketable part of roses, making them unsaleable (Brødsøgaard & Albajes 1999). Although thrips feed on above-ground plant parts, the pre-pupae and pupae live in the litter or soil (Brødsøgaard & Albajes 1999), where they are less affected by chemical control. Biological control with predators is efficient in some crops, such as vegetables (Gillespie 1989; Jacobson et al. 2001; Shipp & Wang 2003; Shipp & Ramakers 2004). Western flower thrips are omnivorous: besides feeding on plant tissue, larvae and adults consume spider mite eggs, predatory mite eggs and whitefly crawlers (Trichilo & Leigh 1986; Faraji et al. 2001; Janssen et al. 2003; van Maanen et al. 2012). Due to all these special characteristics, its control is still a challenge in crops with low damage thresholds, especially ornamentals.

Box 4: Alternative prey: Astigmatic mites

Astigmatic mites such as the mould mite Tyrophagus putrescentiae (Schr.,) (FIGURE 0.5), the dried fruit mite Carpoglyphus lactis (L.) and the flour mite Acarus siro (L.) can be used as alternative prey for predators. These species belong to the Acaridae family, they are cosmopolitan, so they can be used in different crops and in different countries. They are easy to rear and are used as food for predators in mass-rearing systems (Ramakers & van Lieburg 1982).

These astigmatic species inhabit the soil, litter, and decaying vegetable material. Species of the genus Tyrophagus commonly inhabit soils, old hay, mushrooms, house dust, and bird nests (Walter et al. 1986; Mullen & O’Connor 2009). They feed on yeast, and...
alga, nematodes, and possibly also on injured and dead mites (Walter et al. 1986). The mold mite *T. putrescentiae* (Figure 0.4b), is considered a pest of stored products with high lipid and protein content, such as cheeses and grains. It is commonly used as food source in rearing systems of different predatory mites of the family *Phytoseiidae*, and of soil predators of different families, such as *Laelapidae* (Ramakers 1999; Bolckmans & van Houten 2006; Fidgitt & Stinson 2008; Pochubay & Grieshop 2012; Barbosa & de Moraes 2016). *Carpoglyphus lactis* is considered a pest of saccharide-rich stored products such as dried fruits, wine, beer and honey (Hubert et al. 2014). This astigmatid mite species is used to mass-rear *Phytoseius persimilis* (Athias-Henriot) (Bolckmans & van Houten 2006; Fidgitt & Stinson 2008). *Acarus siro* is also considered a pest in stored products such as flour, wheat, and cheese (Solomon 1962). This mite is used to mass-rear *Phytoseius persimilis* from the genus *Amblyseius* and *Neoseiulus*, such as different species such as *Macrocheles* and *Laelapidae* (Ramakers & van Lieburg 1982; Bolckmans et al. 2013).

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Generalist red velvet mite predator
*Balaustium leanderi* performs better on a mixed diet

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Generalist predators have the potential advantage to control more than one pest and to be more persistent than specialist predators because they can survive on different foods. Moreover, their population growth rate may be elevated when offered a mixture of prey species. We studied a generalist predatory mite *Balaustium leanderi* that shows promise for biological control of thrips and whiteflies in protected rose cultures in Colombia. Although starting its life in the soil, this predator makes excursions onto plants where it feeds on various arthropods. We quantified life history parameters of the predator, offering high densities of three pest species: first-instar larvae of *Frankliniella occidentalis*, eggs of *Trialeurodes vaporariorum* and *Tetranychus urticae*, either alone or in combination. The predators completed their life cycle on each diet. The egg-to-egg period was c. 2 months. All eggs were laid in one batch in 1-2 days, indicating a pronounced semelparous reproduction pattern. In general, females reproduced earlier and laid more eggs on mixed diets, and these early reproducers consequently had higher population growth rates than late reproducers. The best diet in terms of egg-to-egg period and juvenile survival was the combination of eggs from whiteflies and spider mites. Spider mite eggs alone and western flower thrips larvae alone were the worst diets. It remains to be investigated whether mixed diets promote the population growth rate of *Balaustium leanderi* sufficiently for biocontrol of whiteflies and thrips in the presence of alternative prey, such as spider mites, to become effective.
**Introduction**

Whether to use generalist or specialist predators for biological control of crop pests is an important and hotly debated topic (Symondson et al. 2002). Most attention has been paid to specialist biocontrol agents, because their dynamics are tightly linked to that of the prey and because of the lower risk of side effects on populations of non-target organisms (Murdoch et al. 1984, 1985; Hassell & May 1986; Murdoch 1994). However, generalist predators are increasingly used for biocontrol (Chiverton 1986; Rosenheim et al. 1993; Settle et al. 1996; Chang & Kareiva 1999; Symondson et al. 2002; Messelink et al. 2012). This also applies to generalist phytoseiid mites that live on plants and feed on various arthropod herbivores (Nomikou et al. 2001; Messelink et al. 2008, 2010), as well as on plant food such as pollen (Nomikou et al. 2002; van Rijn et al. 2002) and on plant fungi, such as spores of mildew (Pozzebon & Duso 2008). Compared to specialist natural enemies, the advantage of using generalist predators is that they can feed on other prey than the target pest, thereby enabling persistence of their populations at high densities, even in periods of low pest density.

A disadvantage of using generalist predators is that they may also attack non-target prey or even other predators (intraguild predation or higher-order predation; Rosenheim et al. 1995; Rosenheim 1998). Although this could potentially disrupt biological control, this is often not the case for intraguild predation (Janssen et al. 2006), but can occur in the case of higher-order predation (Messelink et al. 2011).

Generalist predators may be present on the plants, but they may also live in the soil, yet make foraging excursions onto the plants. In this case, their population size depends on the community of arthropod decomposers and predators in the soil or litter (Settle et al. 1996; Scheu 2001), as well as on the community of arthropod herbivores on the plant canopy. Some species in the genus *Balaustium* (Acari: Erythraeidae) are good examples of species that occur in the litter and on plants (Putman 1969; Childers & Rock 1981; Welbourn 1983; Welbourn & Jennings 1991). Moreover, these species prey on a range of species on plants; they have been observed to feed on fruit-tree red spider mites, *Panonychus ulmi* (Acari: Tetranychidae) (Putman 1969; Cadogan & Laing 1977), eggs and larvae of Lepidoptera and Diptera, aphids and pollen (Hayes 1985). Muñoz et al. (2009) observed that an undescribed species of *Balaustium* (C. Welbourn, pers. comm. 2008) feeds preferentially on immatures of three plant pests: two-spotted spider mites *Tetranychus urticae* (Acari: Tetranychidae), western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) and greenhouse whiteflies, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). The same species was used in this study. These predatory mites are found in the vegetation outside greenhouses of flower cultures on the Bogotá Plateau in Colombia (Torrado et al. 2001) in cold as well as temperate zones (Getiva & Acosta 2004). Provided pesticides are not applied, they can be abundant and are considered as candidate predators for control of several pest species (Muñoz et al. 2009).

In this article, we assess the potential of *Balaustium leanderi* (Fuentes et al. 2014) to feed and reproduce on a diet of spider mites, thrips and whiteflies. We measured prey mortality when exposed to mobile life stages of this predator and we determined the life table parameters on a diet of each of these pest species as well as all possible mixtures. The mixtures were offered because several generalist predatory mites have been shown to reproduce better on mixtures of prey species than on each prey species alone (Oelbermann & Scheu 2002; Messelink et al. 2008), which further increases their capacity for pest control (Messelink et al. 2008, 2010). This research may pave the way for future research on the efficacy of *B. leanderi* as a biocontrol agent and on the role of soil-inhabiting prey (e.g. fungivorous mites) to boost their populations and thereby improve pest control.

**Materials and methods**

**Predator and prey cultures**

Predators and prey were reared under local greenhouse conditions with a daily maximum and minimum temperature of 31.7 ± 3.5 and 11.1 ± 2 °C (mean ± s.d.). The whitefly *T. vaporariorum* was reared on tomato plants (*Solanum lycopersicum*), whereas the thrips *F. occidentalis* was reared on Pelargonium grandiflorum flowers in plastic containers (30 × 16 × 12 cm) with moist paper towels on the bottom to promote humidity. The spider mite *T. urticae* was reared on bean plants. The tomato and bean plants were 2 weeks old at infestation; *P. grandiflorum* flowers were bought weekly from a commercial producer, which sprayed minimal levels of pesticides. The cultures of whiteflies and spider mites were kept in separate compartments in a greenhouse inside cages to avoid contamination.

Predator rearing units consisted of closed plastic containers (18 cm diameter, 20 cm height) with an opening (10 cm diameter) covered with a mite-proof steel mesh for ventilation. To provide a suitable substrate for juvenile development, the bottom of the container was covered with a layer of moist peat (c. 3 cm deep). A disc of paper towel (17 cm diameter) was placed on top of the layer to maintain moisture. A total of 15 adult predator individuals were placed in the container and they were provided with plant material infested with all stages of whiteflies, thrips and spider mites twice per week.

To obtain a cohort of *B. leanderi* eggs, we offered individual young adult females a mixture of the three prey species (Muñoz et al. 2009). To this end, *B. leanderi* adults were placed individually in an arena consisting of a rose leaflet on top of an inverted Petri dish (3 cm diameter), which was used as a base for the leaflet; the petiole was inserted in a water-filled plastic tube (6 cm long, 2 cm diameter) to provide water to the leaflet. Mites were kept on the leaflet inside a ring consisting of an opaque PVC tube.
(1 cm high, 2 cm diameter), with a hole in its wall, closed by a piece of cork underneath which the mites preferred to lay eggs. The ring was closed with plastic wrap (Vinipel) (see **SUPPLEMENTARY MATERIAL 1.1**). After 7-10 days, they had laid at least 50 eggs, these egg batches were transferred to a Petri dish with wet cotton wool (Cadogan & Laing 1977). As soon as the pre-larvae had hatched from the eggs, they were transferred each to a separate arena (the same as described above but now without a hole). In this way, batches of 50 individuals at the onset of the pre-larval stage were obtained. For details of culture methods please see **SUPPLEMENTARY MATERIAL 1.1**.

**Developmental stages, survival and reproduction**

Preliminary observations (K. Muñoz-Cárdenas, pers. obs., 2008) showed that *B. leanderi* reproduces by thelytoky, as is the case in a related species (*B. murorum*, Halliday 2005; *B. nr. putmani*, Hedges et al. 2012). We therefore studied the life cycle of *B. leanderi* with individuals kept singly in an arena (as described above) throughout their life span.

There were seven treatments, three of which involved a daily supply with one of the three prey species, offered in sufficient numbers to prevent prey depletion: 80 spider mite eggs, 80 whitefly eggs or 20 first-instar thrips. Predator larvae were offered half these amounts because of their lower prey requirements. Each treatment was replicated with c. 50 *B. leanderi* individuals, each in a separate experimental unit. We also carried out four treatments involving the following prey combinations based on a replacement design (i.e. half or a third of the amounts of prey offered in the monocultures): (1) 40 eggs of *T. urticae* and of *T. vaporariorum*, (2) 40 eggs of *T. vaporariorum* and 10 larvae of *F. occidentalis*, (3) 40 eggs of *T. urticae*, 10 larvae of *F. occidentalis*, and (4) 30 eggs of *T. urticae*, 30 eggs of *T. vaporariorum* and six larvae of *F. occidentalis*. Each of these treatments was replicated with 15 *B. leanderi* individuals. Predator larvae again received half the amount of prey. Prey mortality was measured to verify that enough prey was offered to the predatory mites (see **SUPPLEMENTARY MATERIAL 1.2** for results).

Spider mite eggs and thrips larvae were replaced daily with fresh prey from the cultures, using a fine paintbrush. Thus, spider-mite eggs were offered free of the silken web produced by *T. urticae*. Whitefly eggs were replaced once per week as follows. Three whitefly females were allowed to lay eggs on rose leaflets in a clip cage in the presence of males for a period of 3 days. The leaflets with eggs were subsequently used as arenas for predatory mites. Excesses of whitefly eggs and honeydew were removed with a small wet cotton swab. We registered the number of days of every stage, the day at which every *B. leanderi* female laid eggs and the day they died.

Effects of diet on life span and pre-oviposition period were analysed using generalized linear models (GLM) with a Poisson error distribution. For fecundity, a quasi-Poisson error distribution was used to correct for overdispersion. The effect of diet on survivorship was analyzed using a Cox proportional hazards model ( Hosmer & Lemeshow 1999; Crawley 2007). To test the effect of diet on the percentage of non-reproductive predatory mites, we performed a GLM with a quasi-binomial error distribution to correct for overdispersion. Treatments were compared using the multcomp package (Hothorn et al. 2008).

**Life table parameters**

As shown below, the reproduction of *B. leanderi* represents a special type of semelparity (Stearns 1976, 1992; Roff 1992), appropriately referred to as ‘pronounced’ semelparity (Hautekeete et al. 2001): all eggs are deposited within a very short time span (also called “big-bang reproduction”; Diamond 1982; Zeineddine & Jansen 2009). Under big-bang reproduction and thelytoky, the life history can be summarized by the following variables: (1) the egg-to-egg developmental time, which then equals the generation time \( T \); (2) the number of eggs produced per reproductive female at age \( T (m_I) \); (3) the survival from egg deposition until reproducing adult \( l_I \).

The latter variable is the product of the survival until adulthood \( l_I \) and the proportion of adults that reproduce \( s_I \). Based on these life table parameters, we calculated the net reproduction rate \( R_0 = l_I m_I \) and the intrinsic rate of increase \( r_m \), which for the special case of big-bang reproduction equals (Carey 1993):

\[
r_m = \ln(R_0) / (T + 1).
\]  

Under big bang reproduction, the variables \( T \) and \( m_I \) can be quantified for each individual separately. Such an individual-based assessment is obviously not possible for the survival probability until becoming a reproducing adult \( l_I \). We therefore provisionally used population estimates of \( l_I \) when estimating the \( r_m \) for each individual, which enabled us to estimate the mean and the variance of \( r_m \).

To explore trade-offs between the net reproduction and the egg-to-egg period, we carried out a regression analysis of individual \( R_0 \) against individual \( T \) and individual \( r_m \) against individual \( T \). To help exploring how the trade-off between individual \( R_0 \) and individual \( T \) influences the relation between individual \( r_m \) and individual \( T \), we plotted \( r_m \) based on the overall mean of \( R_0 \) and \( T \) in relation to \( T \) (necessarily leading to a hyperbolic relation because \( R_0 \) is constant).

The effect of diet on intrinsic rate of population increase was analysed with a GLM with a Gaussian error distribution. To detect trade-offs and possibly other trends in the intrinsic rate of population increase and the net reproduction, these parameters were plotted against the egg-to-egg developmental time. All the statistical tests were done using the software R (2.15.1).
Results

Developmental stages

We observed seven developmental stages (see Supplementary Material 1.1), comparable to the related species *B. putmani* (Putman 1969; Cadogan & Laing 1977) and *B. hernandezi* (Makol et al. 2012): (1) the spherical egg (c. 0.16 mm diameter), initially red but turning dark red later; (2) the red oval prelarva with an orange band (c. 0.2 mm wide, c. 0.3 mm long); (3) the orange-red, six-legged larva; (4) the scarlet, oval and quiescent (legless and sessile) protonymph; (5) the scarlet, oval and eight-legged deutonymph showing three distinctive parallel white dorsal lines; (6) the orange-red tritonymphs (morphologically similar but larger than the protonymph); (7) the bright-red to dark-red adults with four pairs of legs and three white lines on the dorsum (like the deutonymph) (1 mm wide, 1.5 mm long). We found only females, as in *B. murorum* (Halliday 2005).

The overall life span varied significantly with diet (Figure 1.1A; GLM, deviance = 67.7, d.f. = 6,73, \( P<0.0001 \)). The shortest mean life span was found on a mixed diet of eggs of whiteflies and spider mites, the longest when offered thrips larvae. Most of the variation in overall life span was due to differences in the longevity of the adults (Figure 1.1A).

The time from emergence as an adult to the first egg laid (the pre-oviposition period) also varied significantly with the diet offered (Figure 1.1B; GLM, deviance = 123.7, d.f. = 6,47, \( P<0.0001 \)). It was shortest on a mixed diet of whiteflies and spider mites and on a diet of spider mites and thrips, and longest on a diet of thrips or spider mites alone, thrips alone and the combination of all three prey species.

Survival

Survival varied significantly with diet (Figure 1.2; Log rank test = 13.36, d.f = 6, \( P = 0.038 \)). The predatory mites fed with thrips alone or in combination with spider mites showed significantly more mortality compared to the predatory mites fed with the other diets. In general, most mortality occurred during the development from pre-larvae to larvae, which occurs from days 15-30 (Figure 1.2).

![Figure 1.1](image1.png)

**Figure 1.1.** The effect of single prey species and mixtures of species on the life span of *Balaustium leanderi*. Diets consisted of whitefly eggs (W), thrips larvae (T), spider-mite eggs (S), or combinations of these prey. (A) Mean (+ s.e.) life span, including the mean duration of the immature phase indicated by the black bars and the adult longevity indicated by the white bars. **B. putmani** (Putman 1969; Cadogan & Laing 1977) and *B. hernandezi* (Makol et al. 2012): (1) the spherical egg (c. 0.16 mm diameter), initially red but turning dark red later; (2) the red oval prelarva with an orange band (c. 0.2 mm wide, c. 0.3 mm long); (3) the orange-red, six-legged larva; (4) the scarlet, oval and quiescent (legless and sessile) protonymph; (5) the scarlet, oval and eight-legged deutonymph showing three distinctive parallel white dorsal lines; (6) the orange-red tritonymphs (morphologically similar but larger than the protonymph); (7) the bright-red to dark-red adults with four pairs of legs and three white lines on the dorsum (like the deutonymph) (1 mm wide, 1.5 mm long). We found only females, as in *B. murorum* (Halliday 2005).

![Figure 1.2](image2.png)

**Figure 1.2.** Cumulative survivorship of *Balaustium leanderi* predators on different diets. S refers to spider-mite eggs, T to thrips larvae and W to whitefly eggs. Different letters next to the survivorship curves indicate significant differences.
Reproduction

Oviposition occurred within 1 or 2 days, implying big-bang reproduction. Thus, to characterize reproductive effort, it suffices to focus on fecundity per reproductive female (Figure 1.3A). Fecundity varied significantly with diet (GLM, F6.46 = 3.34, P = 0.008). In particular, fecundity on a diet of spider mite eggs was significantly lower than that on a diet of whitefly eggs or of thrips larvae.

The percentage of non-reproductive predatory mites (sT) also varied with diet (Figure 1.3B; GLM, F6.71 = 2.58, P = 0.026). On a diet of whitefly eggs either with or without spider mites, fewer than 10% of the adult predators did not oviposit, whereas on the other diets this was 25% or more. The proportion of non-reproductive preda-

Life table parameters

Using the individual estimates of T and mT and the population estimates of fT, we calculated ‘individual’ rm, their mean and standard error for each diet (Figure 1.4) and found significant dietary effects on rm (GLM, F8.47 = 12.88, P<0.0001). Clearly, a mixed diet of whitefly and spider mite eggs together gave the highest value of rm and a diet of exclusively thrips larvae or spider mites resulted in the lowest values.

The effect of diet on R0 was not tested separately because it essentially converges to analyzing the effect on fecundity (lT being a population estimate). We found a non-significant negative correlation between the generation time T and the net reproductive rate R0 = lT mT (Figure 1.5A; linear regression, d.f. = 1, 52, R² = 0.045, P = 0.12).

Regressing rm against T is not very informative: because T was used to calculate rm, it results in a spurious correlation. Instead, it was thought to be more instructive to see how rm declines with T when rm is calculated following expression (1), using each individual developmental time T (points in Figure 1.5B) and the overall mean R0 (drawn line in Figure 1.5B). Clearly, the data points tend to be above the line for low values of T and below the line for higher values of T. This demonstrates the extent to which the R0-T relation determines the individual rm.

Figure 1.5 illustrates the main points of our life history assessment. First, a diet of thrips larvae or spider mites is the least profitable food in terms of R0 and rm (the data points cluster at the lower right end of the plot). Second, the mixture of eggs of whiteflies and spider mites tend to be the most profitable food source, especially when looking at rm (all data point cluster at the higher left end of the plot; Figure 1.5B).
Discussion

Pronounced semelparity

We show that *B. leanderi* exhibits big-bang reproduction or ‘pronounced semelparity’ (Stearns 1976, 1992; Roff 1992; Hautekeete et al. 2001): they lay all their eggs in 1 or 2 days after a long period of development and die soon thereafter. Big-bang reproduction is widespread among the hard ticks (Acari: Ixodidae), whereas iteroparous reproduction prevails among the soft ticks (Acari: Argasidae) (Sonen-shine 1991). On a continuous scale from ‘pronounced semelparity’ to ‘pronounced iteroparity’ (Hauetteke et al. 2001), many fast-reproducing mites exhibit semelparous reproduction; examples are physoagastic heterostigmatic mites (Bruce & Wrench 1990; Kaliszewski et al. 1995), prostigmatic mites such as eriophyoids (Sabelis & Brun 1996) and tetranychoid mites (Sabelis 1991), and mesostigmatic mites such as phytoseids (Sabelis & Janssen 1994). However, all these mites require a few weeks to produce all offspring and do not immediately die after reproducing (Blommers & van Arendonk 1979). Hence, they do not even come close to the phenomenon of big-bang reproduction as described here for *B. leanderi* and elsewhere for *B. hermandesi* (Makol et al. 2012). Theory on semelparous and iteroparous reproduction predicts that big-bang reproduction is favoured by natural selection when adult survival (up to the next reproductive bout) is predictably very low relative to juvenile survival (Cole 1954; Charnov & Scharffer 1973; Young 1981; Ranta et al. 2002; Zeineddine & Jansen 2009). However, which ecological factors determine predictably low survival in adults of erythraeid predators remains to be elucidated. High soil surface temperatures may cause dramatic mortality, but at least some *Balaustium* spp. seem to resist temperatures up to 48-52 °C and have a high dehydration tolerance (Hedges et al. 2012). Critical factors determining survival of *B. leanderi* clearly need to be investigated.

Effect of diet on life table parameters

In the case of big-bang reproduction, life-history theory predicts the existence of a switch point in the relative importance of net reproduction ($R_0$) and the generation time ($T$) (Caswell 1982). Here, we investigate the existence of such a switch point in *B. leanderi*. The relative contribution of $R_0$ and $T$ to $r_m$ can be assessed from the ratio of $r$-values obtained after a fixed proportional increase ($a$) in $R_0$ (referred to as $r(R_0)$) and in $1/T$ (referred to as $r(T)$):

$$r(R_0)/r(T) = (\ln(aR_0)/(T+1))/([\ln(R_0)/(T+1)] = \ln(aR_0)/\ln(R_0).$$

Expression (2) can be used to calculate the relative contribution of $R_0$ and $T$ to $r_m$ as a function of $R_0$, for equal proportional changes ($a$) in both parameters. However, the changes in $R_0$ and $T$ were not equal in our data set. The $R_0$ on the best diet
CHAPTER 1 | GENERALIST PREDATOR PERFORMS BETTER ON A MIXED DIET

(whiteflies plus thrips) was 21.29 and on the worst diet (thrips) 9.35. Hence, the maximum proportional change in $R_0$ was $a = 2.277 (= 21.29/9.35)$. Likewise, $T$ on the best diet (whiteflies plus spider mites) was 43.75, and on the worst diet (thrips) it was 63.0, resulting in a proportional change of $b = 1.44 (= 63.0/43.75)$. We used these values to calculate the relative contribution of $R_0$ and $T$ to $r_m$ as a function of $R_0$ using:

$$n(R_0/T) = \ln(aR_0)/\ln(R_0).$$

(3)

The curve resulting from this (Figure 1.6) shows that $R_0$ contributes more to increases of $r_m$ for values of $R_0 < 7$, whereas $T$ is more important for higher values. Hence, there is indeed a switch point from $R_0$ being more important to $T$ being more important for realistic values of these parameters.

We subsequently considered all possible diet changes of *B. leanderi* that resulted in an increase of the intrinsic growth rate (hence, changing to a better diet) and calculated the contribution of $R_0$ and $T$ to the increase in $r_m$ using expression (3), using the relative changes in $R_0$ and $T$ for this particular diet shift as estimates of $a$ and $b$. The results are shown as points in Figure 1.6. Our data indeed show that the contribution of $T$ to $r_m$ is higher than that of $R_0$ for higher values of $R_0$, whereas the opposite is true for low values of $R_0$ (Figure 1.6). However, the switch point seems to occur for somewhat higher values of the $R_0$ on the inferior diet than predicted (cf. points and curve in Figure 1.6). The existence of such a switch point has interesting consequences for the evolution of the life-history of *B. leanderi*, which will experience stronger selection for higher net reproduction than for a shorter developmental period on some diets, and the reverse on other diets. This could be verified by setting up an experimental evolution approach with samples from the same population of this predator on different diets.

**Thrips and spider mites as inferior prey**

To explain the low performance on a diet of thrips larvae only, the above analysis indicates that we should focus on the factors that decrease net reproduction of the erythraeid predator ($R_0$). Thrips are known to counter-attack predatory mites, especially their eggs (Faraji et al. 2002a,b; Janssen et al. 2002; Magalhães et al. 2005), and this may offer explanations for the low numbers of eggs found for predators on a diet of thrips. Firstly, the female predators may oviposit normally on a diet of thrips larvae, but counterattacks by the thrips may have resulted in egg mortality, thus falsely giving the impression that the oviposition rate of the predators was low. Second, it is known that predators may retain eggs when eggs run the risk of being preyed upon (Montserrat et al. 2007), and this may have occurred here. Third, it is possible that thrips also injure adult predators, which are therefore unable to produce eggs (for such effects on phytoseiid predators, see Bakker & Sabelis 1989). Furthermore, the low juvenile survival on a diet of thrips larvae (Figure 1.2) may directly result from attacks on predator larvae by thrips larvae. In particular, the larvae of the erythraeid predator are vulnerable because they are similar in size to thrips larvae. The deutonymphs, however, are larger and therefore suffer less from counterattack by thrips larvae. All these possible effects warrant a more detailed study of counterattacking behaviour of the thrips and the impact on the predatory mites.

On the other hand, the decreased performance on a diet of spider mites which led to a $r_m$ as low as the one obtained for the predators fed on thrips (Figure 1.4) was not due to low survival (Figure 1.2) but to the long generation and pre-oviposition time, the low oviposition, and the high number of individuals that did not reproduce. This could indicate that there is a deficiency in the nutrients found in the spider mite eggs: this possible lack of nutrients may have affected the development and reproduction of *B. leanderi*.

**The benefit of a mixed diet**

The major outcome of our study is that the intrinsic rate of population increase was significantly higher when the predators fed on a mixture of whitefly eggs and spider-mite eggs compared to other diets. Such effects of mixed diets have been reported before (Toft & Wise 1999; Oelbermann & Scheu 2002; Messelink et al. 2008; Harwood et al. 2009). Because we used a replacement design for the number of each prey species in the mixtures and because spider-mite eggs are smaller than whitefly eggs
(thus containing less food), the higher reproductive capacity must be due to the nutritional composition of the mixed diet and not due to an increased availability of food. However, the quality of the diet is probably not determined by nutritional content, but also by interference among the prey species, i.e. intraguild predation by thrips larvae, and by prey defences, such as the web of spider mites which protects the eggs, but can also be used as a refuge by thrips larvae. These latter factors were not taken into account in our experiments (since the web was removed) and therefore need to be considered in future experiments.

Clearly, the role of mixed diets for predatory arthropods that are used as biological control agents needs more attention. Mixed diets may boost populations of predators, thereby increasing their impact on pest populations (see also Messelink et al., 2008), even on pests that are difficult to attack by the predators (Messelink et al., 2010). We therefore advocate elaborate testing of dietary effects of all arthropods that are sufficiently abundant in the litter and on the plant and may serve as prey for biological control agents. Moreover, it would be worthwhile to investigate which alternative foods/prey can be manipulated to boost predator populations in the litter layer, thereby increasing their impact on pests in the crop.

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SUPPLEMENTARY MATERIAL 1.1 – Details of culture methods
CHAPTER 1 | GENERALIST PREDATOR PERFORMS BETTER ON A MIXED DIET

Predator cultures

Leaves with prey

Predatory mites

Humid peat

Towel paper

Experimental units

Petri dish lid wrapped with paper towel

Opaque PVC tube fragment

Plastic wrap barrier

Piece of cork

Plastic tube with water

Cork provides oviposition sites

Eggs must be kept on wet cotton

Arenas to assess juvenile development

Experimental units

Experimental units
Balaustium sp.

Life cycle

Eggs

Prelarva

Larva
Balaustium leanderi preying on a greenhouse whitefly adult

Balaustium leanderi preying on western flower thrips larva

Balaustium leanderi adult preying on two-spotted spider mite adult

Egg size
**SUPPLEMENTARY MATERIAL 1.2 – Prey mortality**

**Methods**
As explained in the Methods section of the main text, we provided sufficient amounts of each prey species and stage to allow for maximum predation rates. Each day, the number of surviving prey individuals was assessed per arena. Mortality rates per prey species were first averaged per predator individual per stage and were compared among stages with a mixed effects model with the individual predator as random factor (LME of the nlme package of R). For these comparisons, only the single diets (so not the mixed diets) were used.

**Results**
All mobile stages of the predator consumed prey. Prey mortality rates increased with the developmental stage of predator, irrespective of the prey offered (Figure S1.2.1; Table S1.2.1a).

Because we supplied sufficient amounts of prey, we expected consumption of each prey species in a prey mixture to be lower than with single prey diets due to predator satiation. This was indeed the case for the mixed diet of eggs of whiteflies and of spider mites (Figure S1.2.1), but more eggs of spider mites and more eggs of whiteflies were killed in the presence of thrips larvae than when these eggs were offered alone (Figure S1.2.1; Table S1.2.1b). This may have been caused by thrips larvae acting as intraguild predators on the eggs of whiteflies and spider mites, there-
by increasing overall levels of mortality of these latter two prey. Therefore, we refer to prey mortality rates instead of predation rates throughout the manuscript.

**Table S1.2.1a.** Comparisons among the number of prey consumed by different stages of *Balaustium leanderi* (mixed effects model with repeated measures).

<table>
<thead>
<tr>
<th>Prey</th>
<th>Overall effect and comparisons</th>
<th>df</th>
<th>Likelihood ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whitefly eggs</td>
<td>Overall</td>
<td>2</td>
<td>109.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Larvae-Nymphs</td>
<td>1</td>
<td>77.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Nymphs-Adults</td>
<td>1</td>
<td>24.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Adults-Larvae</td>
<td>1</td>
<td>105.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Thrips larvae</td>
<td>Overall</td>
<td>2</td>
<td>30.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Larvae-Nymphs</td>
<td>1</td>
<td>1.11</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>Nymphs-Adults</td>
<td>1</td>
<td>20.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Adults-Larvae</td>
<td>1</td>
<td>27.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Spider mite eggs</td>
<td>Overall</td>
<td>2</td>
<td>187.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Larvae-Nymphs</td>
<td>1</td>
<td>135.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Nymphs-Adults</td>
<td>1</td>
<td>56.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Adults-Larvae</td>
<td>1</td>
<td>181.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Table S1.2.1b.** Comparison of the prey mortality in treatments with whitefly eggs (W) or spider mite eggs (S) alone and in combination with western flower thrips (T) (linear mixed effects model with repeated measures).

<table>
<thead>
<tr>
<th>Predator stage</th>
<th>Prey</th>
<th>Comparison</th>
<th>df</th>
<th>Likelihood ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larva</td>
<td>Whitefly eggs</td>
<td>W vs. W+T</td>
<td>1</td>
<td>48.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Spider mite eggs</td>
<td>S vs. S+T</td>
<td>1</td>
<td>77.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Nymph</td>
<td>Whitefly eggs</td>
<td>W vs. W+T</td>
<td>1</td>
<td>15.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Spider mite eggs</td>
<td>S vs. S+T</td>
<td>1</td>
<td>44.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adult</td>
<td>Whitefly eggs</td>
<td>W vs. W+T</td>
<td>1</td>
<td>14.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Spider mite eggs</td>
<td>S vs. S+T</td>
<td>1</td>
<td>63.1</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Supplying high-quality alternative prey in the litter increases control of an above-ground plant pest by a generalist predator

Karen Muñoz-Cárdenas, Firdevs Ersin, Juliette Pijnakker, Yvonne van Houten, Hans Hoogerbrugge, Ada Leman, Maria L. Pappas, Marcus V. A. Duarte, Gerben J. Meselink, Maurice W. Sabelis & Arne Janssen

Supplying predators with alternative food can have short-term positive effects on prey densities through predator satiation (functional response) and long-term negative effects through increases of predator populations (numerical response). In biological control, alternative food sources for predators are normally supplied on the crop plants; using the litter-inhabiting food web as a source of alternative food for plant-inhabiting predators has received less attention. We investigated the effect of supplying plant-inhabiting predatory mites with alternative prey (astigmat- ic mites) in the litter on pest control. Predator (Amblyseius swirskii) movement and population dynamics of the pest (western flower thrips) and predators were studied on rose plants in greenhouses. Predators commuted between the above-ground plant parts where they controlled thrips, and the litter, where they fed on alternative prey, although the latter were a superior diet. Predators controlled thrips better in the presence of the astigmatid mites than in their absence. We show that predatory mites can form a link between above-ground pests and the litter food web, and propose that adding alternative prey to the litter of ornamental greenhouses can result in higher predator densities and increased biological control.

Biological Control (2017) 105: 19-26
Introduction

Traditionally, above-ground and below-ground interactions involving the food web associated with plants were studied independently, but it has become clear that these two food webs are connected (A’Bear et al. 2014; Bezemer & van Dam 2005; Gange & Brown 1989; van der Putten et al. 2001). Generalist predators can link above-ground and below-ground food webs by attacking prey in both habitats, thus shaping the composition and structure of the communities (Scheu 2001; Wardle et al. 2004). Such links between spatially coupled food webs may affect the stability of prey dynamics in ecosystems (de Roos et al. 1998; McCann et al. 2005). Here, we investigate whether such predator-mediated links can be used to improve biological control.

Generalist predators are commonly used in biological control of crop pests (Symondson et al. 2002). They can feed on both the target pest and non-pest prey or other food sources (English-Loeb et al. 1993). Supplying alternative food to predators can affect biological control positively by increasing predator survival and reproduction when target pests are scarce (Eubanks & Denno 2000). If populations of predators and prey do not exhibit sustained oscillations, adding extra food will result in an increase in the densities of predators in the longer term through the numerical response. This, in turn, will result in a decrease of the densities of both the pest and the alternative prey (apparent competition; Holt 1977). However, the addition of alternative prey or food may initially decrease predation on target pests when predators concentrate feeding on the alternative food, or because predators become temporarily satiated (Abrams & Matsuda 1996; Holt 1977; van Baalen et al. 2001; van Maanen et al. 2012). These positive effects of adding alternative food on a prey are reminiscent of apparent mutualism (Holt 1977; Abrams & Matsuda 1996).

Whereas apparent mutualism is undesired in biological control, apparent competition is beneficial. For example, Liu et al. (2006) showed better control of spider mites on apple trees in the presence of alternative prey in the longer term (3.5 months), whereas there was no evidence of apparent mutualism in the shorter term. Messelink et al. (2008) found better control of one pest in the presence of another pest, both of which were attacked by the same predator species. Here too, there was no evidence of short-term apparent mutualism, but pest densities of the initial 4 weeks were lacking. A follow-up of this study indeed did show short-term apparent mutualism (van Maanen et al. 2012). Messelink et al. (2008) furthermore showed that the predators performed better on a mixed diet of two pest species, an effect that has not been included in the theory of apparent competition, but which results in even further reductions of prey densities.

Another strategy to improve biological control is to boost the populations of predators not with alternative prey, but with alternative food that does not damage the plant, such as pollen (Calvert & Huffaker 1974; van Rijn et al. 2002; Janssen & Sabelis 2015). However, the application of pollen to a crop can sometimes be risky because pests can also benefit from the presence of pollen (Chitturi et al. 2006; Leman & Messelink 2015; Vangansbeke et al. 2016; but see van Rijn et al. 2002).

We studied a novel approach to improve biological control in ornamental greenhouses, which is supplying alternative prey for predators in the soil/litter layer. Plant-inhabiting predators can feed on alternative prey belonging to the decomposer community, which can feed and reproduce on the available organic material (Settle et al. 1996). The advantage of using the litter food web to supply alternative food is that it leaves no residues on the plant parts to be commercialized, which is desirable in ornamental crops. The risk is that the above-ground target pest and the alternative food are spatially separated, and predators may not commute sufficiently between the above-ground plant parts and the litter, thus either not benefitting fully from the alternative food, or reducing their attacks on the pest. This will even be more risky when the alternative food is of better quality for the predator than the plant-inhabiting pest.

Links between the above-ground and below-ground food webs through generalist predators have been observed in different systems (Moore et al. 2003), such as crops in the field (Settle et al. 1996), forests (Miyashita et al. 2003), grasslands (Hunt et al. 1987; Wardle et al. 2005) and organic farms (Birkhofer et al. 2008). However, there are only a few examples of applications of such links between above-ground and below-ground food webs for biological control (Birkhofer et al. 2008; Halaj & Wise 2002; Settle et al. 1996). Settle et al. (1996) added organic matter in rice fields, thus boosting populations of decomposers and plankton feeders and significantly increasing the abundance of predators and enhancing pest control. In contrast, Birkhofer et al. (2008) showed that increasing decomposer prey densities in wheat fields resulted in increases of populations of herbivores above-ground, and concluded that the predators switched from feeding on herbivores to decomposers (apparent mutualism; Holt 1977).

Hence, contrasting effects have been found of adding alternative prey on biological control. We therefore tested the effect of adding alternative prey to the litter on densities of plant-inhabiting predators and an above-ground plant pest. The study system consisted of rose plants, the pest species Frankliniella occidentalis (Pergande), which cause economic damage in many different crops (Loomans & Mural 1997), the predatory mite species Amblyseius swirskii (Athias-Henriot), a predatory mite used to control thrips and whiteflies (Buitenhuis et al. 2015; Calvo et al. 2015; Messelink et al. 2006; Nomikou et al. 2001; Pijnakker & Ramakers 2008) and several species of soil-inhabiting predators. We assessed whether predators commuted between the above-ground plant parts and the litter layer with alternative prey (astigmatic mites). Subsequently, we tested whether the alternative prey is of supe-
ALTERNATIVE PREY IN THE LITTER HELPS PEST CONTROL IN THE CANOPY

We used two species of astigmatic mites as alternative prey: Tyrophagus putrescentiae (Schrank), the most abundant astigmatic species in the litter of commercial rose crops in greenhouses in the Netherlands (K. Muñoz-Cárdenas, pers. obs.), and Carpoglyphus lactis L., also commonly occurring in rose litter (K. Muñoz-Cárdenas, pers. obs.) and used for mass rearing of A. swirski (Bolckmans & van Houten 2006). Astigmatic mites were provided by Koppert Biological Systems and were reared in cylindrical plastic containers (8 cm diameter, 12 cm high). For T. putrescentiae 10 g of bran (De Halm, Heeswijk, The Netherlands) was added as food; C. lactis cultures received 5 g of bran and 5 g of yeast (De Halm), all once per week. The containers were kept in the laboratory inside styrofoam boxes at 21 °C with wet tissue paper at the bottom to increase humidity.

Movement of Amblyseius swirski

Because A. swirski is mainly known to occur on above-ground plant parts, we tested whether they moved to the litter to feed on alternative prey. In a greenhouse compartment at the University of Amsterdam, 4-week-old rose plants (var. Avalanche; Olij Rozen Int., de Kwakel, The Netherlands) with 3-4 leaves (c. 15 cm high) planted in rock wool were placed inside small insect-proof cages (30 × 30 × 30 cm). Small Petri dishes (2.5 cm) with c. 100 adult females of T. putrescentiae mixed with bran were placed on the rock wool under each plant. There were four treatments: a control without thrips and three treatments with 5, 10 or 25 first-instar thrips larvae on plant leaves. Immediately after releasing the thrips (at 21:00 h), we released five A. swirski females (10-12 days old) per plant on the leaves. Thrips and predators were transferred to the plants using a fine brush. There were two blocks (October 3 and 24, 2012), with three replicates of each treatment per block. Predatory mites in the Petri dishes and on the rock wool were counted 1 h later using a stereo-microscope.

The numbers of predators found under the plant per cage were log(x+1) transformed to stabilize variance, and the transformed data were compared among treatments using a linear mixed effects model (LME, package nlmef of R; Pinheiro et al. 2014) with the number of thrips as fixed factor and block as random factor. Residuals were analysed to check the suitability of the models (Crawley 2013). All statistical analyses were done using R (R Development Core Team 2013).

Effect of alternative prey on thrips densities

Two 10-week-old rose plants (var. White Naomi, Olij Rozen Int, c. 70 cm high) in pots with peat as substrate were placed in mite-proof cages (47.5 × 47.5 × 92 cm) in an acclimatized room at the University of Amsterdam (25 °C, RH 60%, photoperiod L16:D8). Fallen, dried leaves were kept in the pots and flower buds were removed; both are common growers’ practices during the early stages of plants development.
There were two treatments, cages to which *A. swirskii* was added and cages with *A. swirskii* plus alternative prey (*T. putrescentiae*). The experiment was done in two blocks (May and August, 2012), with three replicates (cages) of each treatment per block. Thrips were collected from the rearing unit with a disposable polypropylene pipette tip covered at the wide end with a piece of gauze (mesh 30 μm) and connected to a flexible plastic tube, which was either connected to a pump or used as mouth piece. Air carrying thrips or predators was sucked through the tip, and the tip was subsequently closed at both sides with Parafilm®. A small piece of yarn was taped to the pipette tip to suspend it from a branch of a rose plant. After removing the Parafilm, the thrips could move onto the plants. In the first week, 40 adult thrips were released per cage. During the first, second and third week, 50 adult female *T. putrescentiae*, mixed with 2 g of bran serving as food, were dispersed on the substrate under the plants of the respective treatment. In the third and fifth week, 40 female *A. swirskii* (10-12 days old) were released onto the leaves with a fine brush.

In the second week, 10 leaves/cage were collected, five leaves from the upper part and five from the lower part of the plants to confirm establishment of the thrips. This was repeated in the fourth week, 1 week after the first predator release. The numbers of thrips and the proportion of leaves with thrips damage were scored using a stereo-microscope. The plants did not produce any flowers during the experiment. Because most leaves were already damaged since the first sampling, the proportions of damaged leaves were not analysed. We checked for the presence and identity of *A. swirskii* in all the cages. After 6 weeks, all leaves were collected from each cage and the average numbers of thrips per leaf were scored. These numbers were log(x+0.1) transformed and analysed with a linear mixed effects model (LME) with treatment as fixed factor and block as random factor. Residuals were checked as above.

### Food quality of pest and alternative prey

The quality of astigmatic mites and thrips larvae as food for the predators was assessed by measuring juvenile survival and development and oviposition of *A. swirskii* in the laboratory at the University of Amsterdam. Cohorts of *C. lactis* were prepared by allowing 50-60 females to oviposit on a plastic arena (similar to those used for *A. swirskii*, see “Rearing methods”) with one gram of solid yeast during 24 h (25 °C, RH 70%). Five days later, larvae were taken from these arenas. One day old thrips larvae were taken from the thrips colony. Newly hatched predator larva were transferred each to a separate leaf disc (var. Avalanche) with either eight *C. lactis* immatures, four young first-instar thrips or four *C. lactis* immatures plus two thrips. A surplus of prey was offered, based on reported consumption rates of *A. swirskii* (Bolckmans & van Houten 2006; Messelink et al. 2008). Every day, the juvenile predatory mites were transferred to a new leaf disc with the same food. There were two blocks in time, with 14 or 15 predators (replicates) per treatment in the first block and six in the second block. Survival and the stage of the predator were recorded daily until all mites had reached adulthood or died. Juvenile development and survival were analyzed using time-to-event analysis (Cox proportional hazards, R package coxme; Therneau 2015), with diet as fixed factor and block as random factor. Contrasts among treatments were assessed through general linear hypothesis testing (glht of the lsmeans package with the ‘tukey’ adjustment of P values; Lenth 2016).

The oviposition of adult *A. swirskii* was measured during 10 days on the three diets mentioned above. To obtain adult females, rose leaves on pieces of wet cotton wool in Petri dishes were supplemented with around 50 predator eggs. When the larvae hatched, we daily added around 100 *C. lactis* and/or thrips larvae according to the treatment until copulations of adult predators were observed (after c. 7 days). Twelve females per diet were transferred each to a separate leaf disc with a cotton thread (0.5 cm) as oviposition substrate. They were supplied with either 20 *C. lactis* immatures, eight first instar thrips larvae or 10 *C. lactis* plus four thrips larvae per day. The cotton thread was replaced daily during 10 days and predator eggs were counted. The log(x+1)-transformed numbers of eggs produced per female per day were analyzed with an LME with individual as random factor and the age of the predator and the treatment as fixed factors.

#### Thrips control by *Amblyseius swirskii* with alternative prey

Because soil-inhabiting predators were encountered above (Effect of alternative prey on thrips densities), another experiment was done to specifically study the effect of *A. swirskii* with alternative food on thrips control. We therefore thoroughly washed roots of rose plantlets with running tap water to remove soil mites. Plants (var. Avalanche, 4 weeks old) were planted in clean peat before placing them inside cages in a greenhouse compartment at the University of Amsterdam, where two plants were allowed to grow for 4 weeks, having 10-12 leaves and an approximate height of 30 cm. Litter collected from a commercial greenhouse was sterilized (108 °C, 1 bar, 20 min) to kill mites, moistened (100 ml water / l litter) and placed at the base of the plants.

There were four treatments, each replicated three times: thrips with bran; thrips, *A. swirskii* with bran; thrips with c. 4000 adult female *C. lactis* mixed with all other stages; thrips, *A. swirskii* with bran and female *C. lactis* (same quantities). *Carpoglyphus lactis* was reared on bran, and was released on the litter with c. 100 g of bran for practical reasons. The same quantity of sterilized bran was added to the control treatments. Forty predators (Swirski-mite®) were released on the litter of...
each cage with a mix of bran with or without C. lactis. One week later, each cage received 60 adult thrips using pipette tips (‘Effect of alternative prey on thrips densities’). From the third to the twelfth week, we counted thrips, thrips damage and predators on six leaves (two from the top part, two from the middle and two from the lower leaves). All flowers were collected in plastic containers with alcohol (70%), washed with 70% alcohol on a mesh (100 μm) and the thrips and mites on the mesh were counted under a stereo-microscope. Once per week, we checked 50 ml of litter and bran from each cage for the presence of astigmatic mites and predatory mites under a stereo-microscope. Each week, three of the adult female predators found were identified (Chant & McMurtry 2007; Demite et al. 2014). Predatory mites from the flowers and the litter were identified by Farid Faraji (Mitox Consultants/Eurofins).

The densities of thrips on leaves and in flowers and predators were log(x+0.1) transformed, the proportions of damaged leaves (judged by the presence of feeding scars) were not transformed, and all were analyzed with linear mixed effects models (LME) with the experimental unit (cage) as random factor and time (week) and treatment as fixed factors. The proportion of flowers with heavy thrips damage (>5 feeding scars/flower) were analyzed with a generalized linear model (GLM) with treatment as factor and a quasi-binomial error distribution. Contrasts among treatments and checking of the error distribution were done as above.

Results

A pilot experiment showed that high densities of A. swirskii controlled thrips and decreased the thrips damage of flowers after 4 weeks (SUPPLEMENTARY MATERIAL 2.1). We therefore investigated whether adding alternative food to the litter could boost densities of this predator to sufficiently high levels for thrips control.

Movement of Amblyseius swirskii

Of the five mites released per plant on the above-ground parts, we recaptured on average 37.5% (s.e. 7.2%). The majority of these (1.4 mites/replicate) were found in the Petri dishes with alternative prey; the others were found on the plants. Most probably, the rest of the mites were inside the rock wool, which was not sampled because of its complex structure. If they were indeed there, this would mean that they also moved down. The densities of thrips on the plants had no effect on the movement of predatory mites (FIGURE 2.1; LME: d.f. = 1, $\chi^2 = 6.34$, P = 0.10). These results confirm observations of A. swirskii individuals in the litter in commercial rose greenhouses in which they had been released on the plants (K. Muñoz-Cárdenas, pers. obs.).

Effect of alternative prey on thrips densities

There were no significant differences in the average numbers of thrips per leaf before releasing the predators in the second week (FIGURE 2.2; LME: d.f. = 1, $\chi^2 = 0.17$, P = 0.67) or after predator release in the fourth week of the experiment (FIGURE 2.2; LME: d.f. = 1, $\chi^2 = 0.05$, P = 0.83). Destructive sampling in the sixth week showed significantly fewer thrips per leaf in treatments with A. swirskii plus alternative prey than with A. swirskii alone (FIGURE 2.2; LME: d.f. = 1, $\chi^2 = 6.75$, P = 0.009). Besides A. swirskii, naturally litter-inhabiting thrips similar densities of predators from the family Laelapidae (genera Cosmolaelaps and Stratiolaelaps) were found in the litter in both treatments. Similar results were obtained in a larger scale experiment in the greenhouse (SUPPLEMENTARY MATERIAL 2.1).

FIGURE 2.1. Effect of the density of first instar larvae of thrips (horizontal axis) on leaves of rose plants on the average number (± s.e.) of Amblyseius swirskii moving down from the leaves to Petri dishes with astigmatic mites on the substrate under the plant. There was no significant effect of the number of thrips larvae on predator movement (LME, P>0.05).

FIGURE 2.2. Effect of addition of astigmatic mites (Tyrophagus putrescentiae) as alternative prey in the litter layer under laboratory conditions on average numbers of thrips/leaf (s. a.s.). Data of the second and fourth weeks correspond to samplings of 10 leaves per cage; data of the sixth week correspond to destructive sampling in which the number of thrips were scored on all leaves of each cage. The asterisks indicate a significant difference between treatments (LME, P<0.01).
Food quality of pest and alternative prey
The rate of juvenile development was affected by diet (Figure 2.3a; mixed-effects Cox model: d.f. = 2, \(\chi^2 = 25.1, P<0.001\)); it was similar on diets of C. lactis alone or combined with thrips and lower on a diet of only thrips (Figure 2.3a). Survival was higher in the treatments with C. lactis alone (90%) or combined with thrips (80%) than in the treatment with thrips alone (77%), but these differences were not significant (Figure 2.3a; d.f. = 2, \(\chi^2 = 1.18, P = 0.55\)).

The average oviposition through time of A. swirskii on a diet of C. lactis immatures, first instar thrips larvae or on a mixed diet did also not differ significantly (Figure 2.3b; LME: \(\chi^2 = 3.03, P = 0.22\)).

Thrips control by Amblyseius swirskii with alternative prey
There was a significant effect of the interaction between treatment and time on the densities of thrips on leaves (Figure 2.4a; LME: d.f. = 3, \(\chi^2 = 222.7, P<0.0001\)). This was caused by the densities of thrips remaining low in the treatment with A. swirskii plus alternative food and increasing in the other treatments (Figure 2.4a). Thrips densities on leaves were significantly higher in the treatments with A. swirskii than in the control (Figure 2.4a). The numbers of thrips in the flowers differed significantly among treatments (Figure 2.4b; d.f. = 3, \(\chi^2 = 9.26, P = 0.026\)) and with time (d.f. = 1, \(\chi^2 = 31.4, P<0.0001\)). The numbers of thrips in flowers were significantly lower with A. swirskii plus alternative food than in the control (Figure 2.4b). The interaction between treatment and time had no significant effect (d.f. = 3, \(\chi^2 = 7.66, P = 0.054\)). Alternative prey significantly increased densities of predators (Figure 2.4c; LME: d.f. = 1, \(\chi^2 = 11.8, P<0.001\)). We found A. swirskii in the litter throughout the experiment when it was released together with alternative prey.

The proportion of damaged leaves was significantly affected by the interaction between treatment and time (Figure 2.4d; d.f. = 3, \(\chi^2 = 12.6, P = 0.0057\)). Initially the proportion of damaged leaves was similar in all treatments, but overall, damage lev-
els were significantly lower when *A. swirskii* was released than in the other two treatments (FIGURE 2.4d). The proportion of flowers with heavy thrips damage varied significantly with treatment (GLM, $F_{3,8} = 5.11$, $P = 0.029$). The treatment with *A. swirskii* with alternative food ($12 \pm 8.5\%$ flowers damaged) differed significantly from the control ($73 \pm 11.6\%$). The treatments with *A. swirskii* alone ($32 \pm 13.4\%$) and with alternative food alone ($61 \pm 14.6\%$) did not differ significantly from the other treatments. No litter-inhabiting predators were found in this experiment, confirming that sterilizing the litter and washing the roots of the plants before the experiment had resulted in their exclusion.

**Discussion**

The use of generalist predators for biological pest control has become a commonplace (Janssen & Sabelis 2015; Symondson et al. 2002). Generalists have the advantage that their populations can be maintained in a crop when no pests are present. To date, this was mainly achieved by supplying alternative food on the above-ground plant parts (Adar et al. 2014; Delisle et al. 2015; Duarte et al. 2015; Kumar et al. 2015; Leman & Messelink 2015; Liu et al. 2006; Messelink et al. 2008; Nomikou et al. 2010, 2002; Pijnacker et al. 2016; van Rijn et al. 2002). The current study capitalized on the use of soil or litter-inhabiting arthropods as food for predators. For this strategy to be effective, generalist predators need to forage in both habitats, which was confirmed here for *A. swirskii*. This resulted in better biological control of thrips in the current study, an above-ground pest which passes part of its life cycle in the litter.

A risk of supplying generalist predators with alternative prey is the occurrence of positive effects on pest densities (apparent mutualism; Holt 1977), for which we did not find evidence. Supplementary food or prey can hamper biological control in the short term (Koss & Snyder 2005; Prasad & Snyder 2006), but the presence or addition of alternative food or prey can also lead to high densities of predators (Nomikou et al. 2010). Supplying alternative food in the litter has two advantages. First, no alternative prey or food needs to be added to the above-ground crop parts, which is the marketable part in roses. Second, no pollen needs to be dusted on the plants, which can be risky because thrips can also feed on pollen (Chitturi et al. 2006; van Rijn et al. 2002; Vangansbeke et al. 2016).

Some studies show that the presence of alternative food on plants did not result in yield loss or decrease of plant damage, even if there were decreases in pest numbers and increases in predator numbers (Delisle et al. 2015; Jaworski et al. 2015). In contrast, we found that adding alternative prey for *A. swirskii* to the litter resulted in lower damage of leaves and flowers. When *A. swirskii* and alternative prey were released before thrips infestations, a significant reduction of pest damage was observed (FIGURE 2.4c, FIGURE S2.1.1). We therefore suggest that predators and alternative food should be added to the crop before pest invasion, which will decrease plant damage by thrips. Furthermore, instead of the common practice of putting the litter under the benches with plants, growers could add some humid litter (humidity is essential for survival and development of the alternative prey) at the base of the plants and provide alternative prey in this litter to increase pest control.

A possible disadvantage of adding alternative prey to the litter is that predators will have to commute between the litter and the above-ground plant parts where pests reside. In the case of western flower thrips, this is not a disadvantage because thrips prepupae and pupae live mainly in the litter and *A. swirskii* can attack these stages (K. Muñoz-Cárdenas & M.V.A. Duarte, pers. obs.). Another disadvantage of adding alternative prey is that predators may refrain from attacking the pest when the alternative prey in the litter is of superior quality, as was the case here (FIGURE 2.3). Nevertheless, we found better thrips control with alternative prey in the litter (FIGURE 2.4a). Moreover, there were four times more predators on the above-ground plant parts in the treatment with alternative prey in the litter (FIGURE 2.4b). This suggests that the predators commuted from the litter to the plants and fed on both prey rather than concentrating their attacks on the superior prey in the litter. However, the alternative prey was added at the beginning of the experiments, and their densities may have been reduced towards the end of the experiment, resulting in hungry predators attacking thrips. Clearly, the dynamics and the timing of release of the alternative prey deserve further study, because adding high-quality alternative prey may result in a temporal release of thrips from predation (short-term apparent mutualism; Holt 1977; Abrams & Matsuda 1996).

Another mechanism by which predatory mite populations can increase when feeding on two different prey is through diet supplementation (Marques et al. 2015; Messelink et al. 2008). We did not find evidence for better performance of *A. swirskii* on a mixed diet of thrips and the alternative prey *C. lactis* than on *C. lactis* only (FIGURE 2.3). Instead, the juvenile survival and developmental rate was highest on diets containing *C. lactis*, irrespective of the addition of thrips (FIGURE 2.3a). In agreement with this, we found the highest densities of predators when this alternative prey was present (FIGURE 2.4b). This confirms that *C. lactis* is a better food source for *A. swirskii* than are thrips larvae.

It is not obvious that plant-inhabiting predators such as *A. swirskii* move down to the litter to feed on alternative prey. Buitenhuis et al. (2010) reported that *A. swirskii* moved down to the soil to disperse; we found that they move to this habitat provided there are astigmatic mites in the litter on which they can feed (FIGURE 2.1). Possibly, the presence of alternative prey in the litter caused *A. swirskii* to forage more there, also attacking thrips pre-pupae and pupae (K. Muñoz-Cárdenas & M.V.A. Duarte,
pers. obs.). Other litter-inhabiting predatory mites can also feed on these thrips stages and on the alternative prey in the litter. Therefore, further experiments should assess the effect of these litter-inhabiting predators on pest populations and their interactions with plant-inhabiting predatory mites, especially because it has been shown that intraguild predation between plant-inhabiting and litter-inhabiting predators can occur (Messelin & van Holstein-Saj 2011) and might disrupt biological control (Vance-Chalcraft et al. 2007; Rosenheim et al. 1995; but see Janssen et al. 2006).

In conclusion, our results demonstrate that links can be established between above-ground plant pests and the litter food web and that such links can benefit plant-inhabiting predators, resulting in an increase of predator densities and enhanced biological control. This confirms the importance of considering connections between above-ground and below-ground food webs associated with plants (A’Bear et al. 2014; van der Putten et al. 2001), also for applied purposes.

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SUPPLEMENTARY MATERIAL 2.1

Thrips control with high densities of Amblyseius swirskii

We tested whether releasing high densities of A. swirskii (1000 mites/m²) of greenhouse, commercial recommendation is 100 mites/m²), could satisfactorily control thrips in roses. If this were the case, it would be worthwhile to investigate which type of alternative food can be used to boost the density of this predatory mite species to sufficiently high levels for thrips control.

Methods

Rose plants (var. Avalanche) were placed in insect-proof cages with a wooden frame (140 × 160 × 80 cm) in a greenhouse compartment at Koppert Biological Systems in Berkel en Rodenrijs, The Netherlands (22.2 ± 2.5 °C, RH 61.8 ± 10.6%). Each cage was considered an experimental unit and contained six rose plants (Avalanche, c. 18 weeks old) in pots (26 cm diameter, 25 cm high) with peat as potting substrate (50% coco peat, 15% coconut peat, 35% frozen black peat; Jongkind grond BV, Aalsmeer, The Netherlands). Plants were watered with a drip irrigation system. According to commercial practice, rose stems with shoots and buds were bent to allow more shoots to develop. Because there was some risk of previous contamination of the plants, around 10 Phytoseiulus persimilis (Spidex®, Koppert Biological Systems) and 50 Encarsia formosa (En-strip®, Koppert Biological Systems) were released per plant to control spider mites and whiteflies, respectively.

Thrips were collected from the stock colony of Koppert Biological Systems. To release 1000 A. swirskii/m², we took 10 ml of the commercial product Swirski-mite® (containing 50,000 predators / 500 ml of bran). In each cage, 130 adult thrips were released in the first and second week. There were two treatments: one with only thrips and one with thrips and 1000 adult female A. swirskii, released in the third week. There were two trials with two replicates (cages), each lasting for 30 days (March to May 2011). Plants were sampled destructively at the end of the experiment. All leaves in the lower and upper stratum were counted, as well as the buds and flowers of all the plants. We counted the thrips and mites on 15 leaves of the upper stratum and 15 leaves of the lower stratum and in all flowers and buds under a stereo-microscope.

The numbers of thrips in the flowers and on the leaves (√x transformed) were compared between treatments using LME, with treatment as fixed factor and trial as random factor. The distribution of the residuals was checked for normality and treatments were compared by combining factor levels.
**Results**

Significantly lower densities of thrips were found on the leaves in the cages with predatory mites than in the cages with only thrips (average ± s.e. = 0.058 ± 0.039 vs. 0.24 ± 0.10 thrips/leaf; LME: d.f. = 1, $\chi^2 = 4.04$, $P = 0.044$). The difference in the average densities of thrips in the flowers and buds was more pronounced than that on leaves (with predators: 2.23 ± 0.59 thrips/flower, without predators: 13.2 ± 5.0; LME: d.f. = 1, $\chi^2 = 6.12$, $P = 0.013$). This also resulted in less damage to the petals of the flowers (Figure S2.1.1). We found 157.2 (± 55.4) *A. swirskii* on the plants per cage.

**Thrips control by predatory mites with alternative prey**

To further test the effect of adding alternative food on thrips densities (cf. *Effect of alternative prey on thrips densities* in the main text), we performed a greenhouse experiment. This experiment was performed on a larger scale than the one in the main text. The plants used here were older and had been sprayed with chemicals. So, we wanted to test whether under these conditions (similar to commercial rose production) we could observe a similar effect of alternative prey on thrips populations as in the experiment performed in laboratory conditions and presented in the main text.

**Methods**

Insect-proof cages (120 × 90 × 80 cm) were placed on tables in a greenhouse compartment at Wageningen UR Greenhouse Horticulture (Bleiswijk, The Netherlands) from September to December 2012 (20 °C, RH 85%, photoperiod L18:D6 artificial light: 10,000 lux). Each cage was considered an experimental unit and contained three 1-year-old rose plants (var. Red Naomi; Schreurs, De Kwakel, The Netherlands). Each plant was placed in a plastic dish (40 cm diameter), which was placed in a plastic tray. Plants were sprayed before the experiment, once with a 0.25% solution of Dodemorf (Metaltox, BASF) against powdery mildew (3 weeks before the experiment) and twice (3 and 2 weeks before) with a 0.028% solution of fonicamid (Tepeki, Belchim) to kill whiteflies present on the plants. The plants were rooted in rockwool strips. Stems with shoots and buds were bent to allow development of more shoots. Flowers that developed from the bent shoots near the soil were not removed but were kept in order to facilitate the establishment of thrips.

As in the previous experiment, *P. persimilis* and *E. formosa* were released preventively. Thrips were collected from rose flowers in a greenhouse of the experimental station using a pipette tip (cf. *Effect of alternative prey on thrips densities*). The tips were inserted into the substrate under the plants and the wide end was opened to release the thrips. *Amblyseius swirskii* (*Swirski-mite*, Koppert Biological Systems) were collected on sweet pepper leaf discs (2.5 cm diameter, 15 females/leaf disc), which were put on the litter layer. Litter was collected from a commercial greenhouse in Stompwijk (The Netherlands). In an attempt to remove arthropods, litter was incubated in Berlese funnels for 7 days before the experiment. Subsequently, 90 g of litter was moistened with 60 g of water and was added at the base of each plant. *Tyrophagus putrescentiae* were obtained from Koppert Biological Systems and added to the litter (Table S2.1.1). There were four treatments: only thrips, thrips + alternative prey, thrips + *A. swirskii*, and thrips + *A. swirskii* + alternative prey. Each treatment was replicated three times. The schedule of thrips and mite releases is shown in Table S2.1.1.

Every week from the 6th until the 9th week, 10 leaves from the upper stratum, 10 from the middle and 10 from the lower stratum were taken randomly per cage. From these 30 leaves collected, we counted the leaves damaged by thrips for each cage but we did not count the thrips or predators on the leaves. We checked that *A. swirskii* was present in all treatments in which they were released but they were not counted. In the 10th week, all leaves were collected and the numbers of thrips and predators were scored under the stereo-microscope. Additionally, the number of leaves with thrips damage was scored. Flowers and buds were sampled as explained in the main text (cf. *Thrips control by Amblyseius swirskii with alternative prey*).

The proportions of damaged leaves from the 6th to the 9th week were arcsin $\sqrt{x}$ transformed, and compared using LME as explained in the main text, with experimental unit (cage) as random factor and treatment and time as fixed factors. Proportions of damaged leaves from the destructive sampling in the 10th week were analysed using a GLM with a quasibinomial error distribution with treatment as fixed factor.
factor. The numbers of thrips on leaves were √x transformed and analysed with a GLM with a Gaussian error distribution. The numbers of thrips in flowers and buds were analysed with a GLM with a Poisson error distribution. The numbers of predators per cage were compared with a GLM with treatment as fixed factor and a quasi-Poisson error distribution.

Results

There was a significant effect of the treatments on the proportion of leaves with thrips damage from the 6th until the 9th week (Figure S2.1.2a; LME: d.f. = 3, χ² = 11.2, P = 0.01), with the lowest proportion of damaged leaves in the treatment with *A. swirskii* and alternative prey (Figure S2.1.2a). In the 10th week, the proportion of damaged leaves was lowest in the treatment of *A. swirskii* with alternative food but there was no significant effect of treatments (FIGURE S2.1.2a; right-hand data points, GLM: F3,8 = 1.75, P = 0.23). Densities of thrips on leaves were low (FIGURE S2.1.2b) and did not differ significantly among treatments (GLM: F3,8 = 2.61, P = 0.12). In contrast, the numbers of thrips in flowers and buds were high (FIGURE S2.1.2b) and differed among treatments (GLM: d.f. = 3, χ² = 40.1, P<0.0001), with the lowest densities in the treatment with *A. swirskii* with alternative food (FIGURE S2.1.2b). There was a trend of higher densities of *A. swirskii* per cage in the treatment in which the alternative prey was added than in the treatment in which *A. swirskii* was released alone (71.3 ± 27.4 vs. 16.7 ± 4.8; GLM: F1,4 = 6.15, P = 0.068).

In all treatments, we found on average two litter-inhabiting predators from the family Laelapidae per open flower close to the litter. Presumably, these mites came from the litter collected in the commercial greenhouse, indicating that removing the litter-inhabiting arthropods in the Berlese funnels was not successful. These predators may have affected the control of thrips.
Alternative food for litter-inhabiting predators decreases pest densities and above-ground plant damage

Karen Muñoz-Cárdenas, Diana Rueda-Ramirez, Firdevs Ersin, Farid Faraji & Arne Janssen

Biological control using generalist predators is successful in many crops. Such predators feed on pests and food or alternative prey that do not cause economic damage to the plants. Adding food for predators to a crop has a positive effect on their numerical response. Thus, more predators are available to potentially attack the pest, reducing their densities (apparent competition). However, in the short term, the addition of alternative food may decrease the attack of pests because the predators are satiated or because they preferentially feed on the alternative food (apparent mutualism). Such positive effects of alternative food on pest densities may occur repeatedly when populations of pests or of alternative food fluctuate. We investigated whether providing generalist litter-inhabiting predators with alternative prey in the litter can increase control of thrips, Frankliniella occidentalis. The larvae of this pest feed on above-ground plant parts, causing significant damage, but prepupae and pupae live in the litter layer. Densities of thrips and damage on above-ground plant parts were significantly reduced when supplying litter-inhabiting predators, predominantly Cosmolaelaps n. sp., with alternative food. Subsequently, Cosmolaelaps n. sp. was tested separately; this species alone also reduced thrips densities and damage significantly when it was supplied with alternative food. Concluding, litter-inhabiting predators can significantly reduce above-ground plant damage when supplied with alternative food in the litter layer.
**Introduction**

Generalist predators can efficiently control pest populations in crops (Fleschner 1959; Ramakers 1990; Symondson et al. 2002; van Rijn et al. 2002; Messelink et al. 2008, 2010). They can act as a link between above-ground and below-ground food webs (Scheu 2001; Muñoz-Cárdenas et al. 2014, 2017) when they feed on above-ground plant pests but also on below-ground prey such as herbivores and detritivores (Settle et al. 1996; Muñoz-Cárdenas et al. 2017). Alternative foods allow generalist predators to survive and reproduce when densities of the target pest are low (Settle et al. 1996; Wäckers et al. 2005; Liu et al. 2006), and they can also result in lower pest densities in the long term due to a negative indirect interaction between the pest and the alternative food. In short, this interaction works as follows: the addition of alternative food results in higher densities of the predators, resulting in lower pest densities of the pest (so-called apparent competition; Holt 1977). However, adding food for predators to the crop can also result in higher pest densities (so-called apparent mutualism; Holt 1977; Abrams & Matsuda 1996), and this is obviously detrimental for biological control (van Maanen et al. 2012). The mechanism behind this is that in the short term, predators might concentrate their attacks on the alternative prey or become satiated by feeding on this prey, freeing the target pest from attacks. Concluding, adding alternative food for predators may not always result in better pest control and this needs to be verified for each combination of predator and prey species and alternative food type.

Several studies in vegetable crops have demonstrated the benefits of additional herbivorous prey or supplementary food for plant-inhabiting predators on biological control of above-ground pests (Collyer 1964; Karban et al. 1994; van Rijn et al. 1999, 2002; Liu et al. 2006; Messelink et al. 2008). In contrast, biological control in ornamentals has been less successful because of the much lower damage thresholds. This could in theory be solved by adding more alternative food, resulting in higher predator populations. The obvious danger of adding more alternative food are that it may result in decreased pest suppression (apparent mutualism). Another problem could be that consumers might not be prepared to accept a product with alternative food or alternative prey on it, even when these do not damage the product. Thus, a solution would be supplying alternative food to increase populations of potential biological control agents away from the marketable part of the crop, for example in the litter or in the parts of the plant that are not sold.

A few studies have investigated the effect of adding alternative food below-ground to control pests on the above-ground plant parts. Some of these studies showed a positive effect on biological control, other studies showed negative effects, reminiscent of apparent mutualism (Settle et al. 1996; Birkhofer et al. 2008; Muñoz-Cárdenas et al. 2017). In a previous study, we show that a generalist predatory mite [Amblyseius swirskii (Athias-Henriot)] can act as a link between above-ground and below-ground food webs by feeding on an above-ground plant pest [Frankliniella occidentalis (Pergande)] but making excursions to feed on alternative prey (astigmat-occidentalis) below-ground (Muñoz-Cárdenas et al. 2017). Adding this alternative prey resulted in improved biological control of thrips and higher densities of predators on the above-ground plant parts. In some experiments, we observed that predators that naturally inhabit the litter also fed on the alternative prey and on pupae of F. occidentalis (western flower thrips), which occur in the litter (Muñoz-Cárdenas et al. 2017). In this paper, we investigate the suitability of these predators for biological control of thrips in roses.

Some predatory mites from the soil and litter are known to play an important role in controlling insect pests with edaphic stages such as thrips (Wu et al. 2014), especially when they are released in combination with other biological control agents (Gillespie & Quiring 1990; Premachandra et al. 2003; Wu et al. 2016). In some cases, releases of commercially available soil-inhabiting predators have resulted in satisfactory reduction of thrips populations, but only when released in high numbers (Wielhoff et al. 2004). Such high densities of soil-inhabiting predators could also be obtained by adding alternative prey to the soil or litter, thus increasing predator populations. However, the effect of adding alternative prey on the population dynamics of litter-inhabiting predators and above-ground pests has not been studied. The goal of this study is to assess whether biological control of thrips can be increased by supplying litter-inhabiting predators with alternative prey. We collected litter with predatory mites from a commercial greenhouse and tested the two most common predatory mites for their capacity to feed on the alternative prey and on thrips. Subsequently, two experiments were done to assess the capacity of the predators to control thrips damage on rose plants; one experiment with multiple predatory mite species and one with the most promising species.

**Materials and methods**

**The experimental system**

We studied rose plants, which are often damaged by the western flower thrips (Park et al. 2002; Manners et al. 2013). Above-ground plant parts of many crops are affected by this thrips species, which spends part of its life cycle in the soil or litter (Tommasini & Maini 1995; Lewis 1997). The alternative prey used here was *Carpoglyphus lactis* (L.), an astigmatic mite commonly found in the litter of commercial rose crops in The Netherlands (Muñoz-Cárdenas et al. 2017). These mites are not considered a pest in roses and are used for mass-rearing of predatory mites (Ramakers & van Lieburg 1982; Bolckmans & van Houten 2006).
Cultures
Rose plantlets were bought when they were 4 weeks old and had 4-5 leaves (Rosa sp. var. Avalanche) (Olij Rozen Int. BV, De Kwakel, The Netherlands). Because these plants had been kept in a commercial greenhouse, there was a risk that they were contaminated with pests. In order to eliminate these, we removed the leaves and washed the stems and roots under running tap water. Subsequently, the remaining plant parts were dried and planted in clean peat. They were allowed to regrow for 4 weeks in a greenhouse compartment (22 °C, RH 70%, photoperiod L14:D10) inside insect-proof cages to avoid new contaminations. The regrown plants were inserted in rock wool strips (Grodan®Delta: 40 x 6 x 28), were watered twice a week, and macro- and micronutrients were applied dissolved in water once a week (0.5 g N-P-K and 0.5 g micronutrients mix/200-500 ml of water/plant). Before the first experiment, P. per- similis (Spidex®, Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands) was preventively released (20 per cage) to avoid contamination with spider mites.

Frankliniella occidentalis was taken from the stock colony of Koppert Biological Systems. They were reared (at 23 °C, RH 65%) using a method adapted from Loomans & Mural (1997). In short, thrips were reared on fresh bean pods inside plastic containers (8 x 8 x 12 cm) with openings covered with mite-proof mesh (30 μm) for ventilation. Around 200 adult thrips were introduced in a rearing unit and allowed to oviposit in two bean pods for 1 or 2 days to obtain cohorts to be used in the experiments. Before use, the pods were washed with soap, rinsed and dried to remove residues of pesticides. Typha latifolia pollen was provided as food for the thrips. This pollen was collected during spring from plants growing on the campus of the University of Amsterdam. Carpglyphus lactis were provided by Koppert Biological Systems and were reared as explained in Muñoz-Cárdenas et al. (2017), inside cylindrical plastic containers (8 cm diameter, 12 cm high) to which 5 g of bran plus 5 g of yeast were added as food once per week. To facilitate the release of C. lactis in experiments, we counted C. lactis per mg of the mixture of bran and yeast from the rearing unit and added the appropriate weight of the mixture to the experimental units to end up with the desired numbers of C. lactis females per experimental unit.

Litter-inhabiting predators that benefit from alternative prey
In previous experiments, we observed that predators inhabiting the moss and litter in commercial greenhouses might play a role in controlling thrips when alternative food (C. lactis) was added to the litter (Muñoz-Cárdenas et al. 2017). For this reason, we did not collect the mite fauna directly from the samples taken in the commercial greenhouses, but we selected mites that could reproduce on C. lactis. To this end, we collected five samples of 1.5 l of various materials from different rose greenhouses in The Netherlands: 1) litter covering the soil in Amstelveen in October, 2011 and 2) in December, 2013; 3) litter from Stompwijk, July 2012; 4) moss growing on the surface of rock wool strips where plants were grown from Aalsmeer, January 2013; and 5) old, open flowers from Amstelveen, July 2013. These latter flowers are non-marketable, and their stems are bent as a common practice. When those flowers develop they can be found touching the litter layer, thus serving as bridges between the above-ground plant parts and the litter. Samples were placed on top of humid rock wool (Grodan®Delta: 28 x 6 x 28) in a plastic tray and spread c. 100 g of food (c. 4000 C. lactis females plus bran) on top of the samples. Each tray was placed inside a mite-proof cage (47.5 x 47.5 x 92 cm) in a greenhouse compartment. After 10 days, we collected each sample and placed it in a Berlese funnel for 5 days.

The predatory mites were collected in vials with alcohol 70% for taxonomic identification. Most predators were found in the litter samples; only few were found in the moss and open flowers, we therefore refer to them as litter-inhabiting predators. Predatory mites were mounted on glass slides with Hoyer’s medium. Subsequently, they were observed under a phase contrast microscope and taxonomic keys were used for identification (Krantz & Ainscough 1990; Lindquist et al. 2009). Adult females were identified to species, based on the original and complementary descriptions. An extra sample (1.5 l of litter) was taken from the greenhouse in Amstelveen in December 2013 in order to extract five mites to set-up cultures of the most common predatory mites (Cosmolaelaps n. sp. and Proctolaelaps pygmaeus) in the laboratory (22 °C). Rearing units consisted of plastic containers (125 ml) with humid plaster of Paris mixed with coal at the bottom (Lesna et al. 2009). Peat mixed with autoclaved rose litter was added on top of the plaster. We introduced 50 adult females of Cosmolaelaps n. sp. or P. pygmaeus to each rearing unit. The plastic containers with the colonies were placed inside a tray with water to increase humidity, the tray was covered with another plastic tray to shield from direct light. A mix of bran with fresh yeast and C. lactis (30 g) were added to each container as food for the predators once per week. We collected around 10 individuals from the colonies, three times during this study for identification.

Predation of Frankliniella occidentalis and Carpglyphus lactis by Cosmolaelaps n. sp. and Proctolaelaps pygmaeus
This experiment was performed to assess whether the litter-inhabiting predatory mites Cosmolaelaps n. sp. and P. pygmaeus feed on thrips and the alternative prey C. lactis. Twenty P. pygmaeus females and 30 Cosmolaelaps n. sp. females were taken from the rearing units and starved individually by placing each in a vial (3 cm diameter, 4 cm high) with a moistened bottom of plaster of Paris mixed with charcoal (Lesna et al. 2009). The next day, we added 10 protonymphs of C. lactis to the vials of one group of females of each species (10 females of P. pygmaeus and 15 females of Cosmolaelaps
n. sp.), and we added three thrips pre-pupae to the vials of second groups of predators (same numbers as above). We added pieces of dried leaves to simulate the structure of the litter. One day later, we scored how many individuals of each prey were eaten. This experiment was carried out at 25 °C, RH 60%, and photoperiod L16:28.

Thrips control by litter-inhabiting predators

Litter was collected in plastic bags from a commercial greenhouse (Amstelveen, April 2014) and was kept in a cold room (4 °C) until the beginning of this experiment in May 2014. This litter harboured different predator species inhabiting the commercial greenhouse. In a greenhouse compartment at the University of Amsterdam, experimental units consisted of two rose plants in rock wool in a mite-proof cage (47.5 x 47.5 x 92 cm). During the first week of the experiment, 1 l of litter moistened with 100 ml of water was placed at the foot of the rose plants. Around 2 h later, c. 100 g of bran with alternative prey (c. 4000 C. lactis females) was dispersed on the litter of three of the experimental units; six other cages received bran without alternative prey. The three treatments (predators with or without alternative prey and the control with only thrips) were replicated three times (three cages). Subsequently, for the treatment with alternative prey, 30 adult females of Cosmolaelaps n. sp. were transferred from the colonies to plastic Petri dishes with bran and C. lactis with a fine brush. For the treatment without alternative prey, three Petri dishes were prepared with only bran and 30 Cosmolaelaps. For the control treatment, we removed all the visible predatory mites from the litter with an aspirator and we prepared Petri dishes with only bran. The content of the Petri dishes was subsequently dispersed on the litter inside the three cages with alternative prey and in six cages without alternative prey. In the second week, the rose plants were infested with 60 adult thrips/cage, which were collected from the colonies using an aspirator made of a disposable polypropylene pipette tip covered at the wide end with a piece of gauze (mesh 30 μm) (Muñoz-Cárdenas et al. 2017). This wide end was connected with a long, flexible piece of tubing to a pump. Thrips were sucked up through the tip, which was subsequently closed with Parafilm® (Sigma-Aldrich, The Netherlands). To release the thrips, we taped a piece of yarn to the pipette tip and suspended it from a branch of the plant. Subsequently, the Parafilm was removed and thrips were free to infest the plant.

From the third to the tenth week, five leaves were collected from each of three plant strata (higher, medium and lower) and thrips larvae and adults were counted using a stereo-microscope. The total number of leaves and the number of leaves with thrips damage were also recorded. As soon as the plants produced flowers (after 4 weeks), these were collected once every 2 weeks, until the tenth week, placed in plastic containers with alcohol (70%), washed with 70% alcohol on a mesh (100 μm) and the thrips remaining on the mesh were counted under a stereo-microscope. Flowers were also checked for damage under the stereo-microscope. We recorded a flower as damaged when it had more than five scars on petals and sepals (each scar of around 2 mm diameter). We selected this number and size of scars because previous studies showed that this area of damage is not visible to the naked eye (Muñoz-Cárdenas et al. 2017). The presence of astigmatic mites and predators in the litter was confirmed by checking 50 ml of humid litter with bran from each cage under a stereo-microscope every 2 weeks (from the fourth to the tenth week), but mites were not counted. On the tenth week we identified up to 10 predatory mite adults from the litter samples per cage.

The average numbers of thrips per leaf were \(\log(x+0.1)\) transformed and analysed with a linear mixed effects model (package lme of R; Pinheiro et al. 2014; R Development Core Team 2015) with replicate (cage) as random factor and time and treatment as fixed factors. The significance of factors and their interaction was determined by comparing models with and without them with the anova function of R. Contrasts between treatments were assessed with a general linear hypothesis test (glht function of the package lsmeans of R; Lenth 2018) with the Tukey method for correction of multiple comparisons. The proportions of damaged flowers and the average numbers of thrips per flower were \(\sqrt{x}\) transformed. The proportions of damaged leaves were analysed using a similar model as above on untransformed proportions.

Thrips control by Cosmolaelaps n. sp.

For this experiment, we excluded predators from the litter (from a commercial greenhouse in Amstelveen, September 2014) by sterilizing it (108 °C, +1 bar pressure, 20 min), thus avoiding possible negative effects of competition among predator species on biological control due to competition or intraguild predation among predator species (Rosenheim et al. 1995; Messelink & van Holstein-Saj 2011). We used Cosmolaelaps n. sp. as litter predator because it inhabits the litter in greenhouses and it feeds on prepupae of thrips. Moreover other species of this genus have also shown potential for thrips control (Furtado et al. 2015). This experiment was performed from September to November 2014 (22 °C, RH 85%, photoperiod L16:D8) at the University of Amsterdam. There were three treatments: Cosmolaelaps n. sp. with alternative prey (C. lactis + bran in sterilized litter), Cosmolaelaps n. sp. without alternative prey (only bran in sterilized litter), and a control with only thrips (60/cage) with bran in sterilized litter. Each treatment was replicated three times (cages).

Besides the sterilization of the litter, the experimental set-up and the samplings were similar to those in the experiment above. The alternative prey and the predators were released as above, for the treatment with alternative food (c. 100 g of bran + 4000 C. lactis females + 30 Cosmolaelaps n. sp.) and for the treatment without alternative food we used bran without C. lactis; thrips were released as above (second week, 60 thrips/cage). Sampling was similar as above, but we sampled nine leaves
per week per cage, three leaves per stratum (higher, medium and lower plant parts). As soon as the plants produced flowers (week 4), these were sampled every week. Here, we identified and counted predators per 50 ml of litter sample every week. We checked for the presence of C. lactis but we did not count the mites.

The average numbers of thrips and damage on leaves and flowers were analysed as above. The numbers of predators per sample were log(x+1) transformed and analysed as above for thrips.

Results

Litter-inhabiting predators that benefit from alternative prey

We found two species of thrips predators benefiting from the alternative prey added to the greenhouse litter. Cosmolaelaps n. sp. naturally occurs in the litter layer in rose greenhouses and was found in high percentages in all samples (Table 3.1). Stratiolaelaps scimitus was found in low numbers in two samples (Table 3.1), this predator is commercially available in The Netherlands, possibly, it was present because of previous releases by the grower (K. Muñoz-Cárdenas, pers. comm.). The other species encountered are not known to feed on thrips but on fungi, nematodes, collembolans, mites, insects other than thrips, or different combinations of these groups (Table 3.1).

Predation of Frankliniella occidentalis and Carpoglyphus lactis by Cosmolaelaps n. sp. and Proctolaelaps pygmaeus

Cosmolaelaps n. sp. fed on C. lactis immatures (average ± s.e.: 8.2 ± 0.4 per day) and F. occidentalis pre-pupae (1.3 ± 0.2 per day). Proctolaelaps pygmaeus did not feed on F. occidentalis pre-pupae but it fed on C. lactis immatures (3.5 ± 0.5 per day).

Thrips control by litter-inhabiting predators

Thrips densities on leaves differed significantly through time among treatments (Figure 3.1a; LME: interaction of treatment with time; χ² = 25.5, d.f. = 2, P<0.0001). This interaction was caused by the numbers of thrips increasing through time without alternative food and in the control and remaining low when predators were supplied with alternative food. Overall, the two treatments without alternative prey differed significantly from the treatment with alternative prey (Figure 3.1a; contrasts after LME). Thrips densities in the flowers did not differ significantly among treatments (Figure 3.1b; LME: χ² = 4.8, d.f. = 2, P = 0.09). There was no significant effect of the interaction of treatment with time (LME: χ² = 1.97, d.f. = 2, P = 0.37).

The proportion of damaged leaves varied significantly through time (LME: χ² = 5.70, d.f. = 2, P = 0.17), but there was no significant effect of the interaction of treatment and time (LME: χ² = 2.22, d.f. = 2, P = 0.33; Figure 3.1c). The proportions of damaged leaves differed significantly among treatments (LME: χ² = 26.7, d.f. = 2, P<0.0001). The proportions of damaged leaves were lower in the treatment with alternative prey than in the two other treatments (Figure 3.1c; contrasts after LME).

The proportion of damaged flowers differed significantly among treatments (LME: χ² = 13.7, d.f. = 2, P = 0.0011). The proportion of flowers with thrips damage was

<table>
<thead>
<tr>
<th>Habitat/Sample</th>
<th>Species</th>
<th>%a</th>
<th>Feeding habits (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter/A</td>
<td>Benoinyssus sp.</td>
<td>9.3</td>
<td>Fungi / Yeast (1)</td>
</tr>
<tr>
<td></td>
<td>Cosmolaelaps n. sp.</td>
<td>14.8</td>
<td>Collembolans / Insects: Thysanoptera / Mites</td>
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<tr>
<td></td>
<td>Gaoaelaelaps queenslandica</td>
<td>8.6</td>
<td>Nematodes (2)</td>
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<td>Gamaseilodes sp.</td>
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<td>Laeloseius subteraneus</td>
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<td>Collembolans / Insects: Hemiptera and Diptera / Mites / Nematodes (5)</td>
</tr>
<tr>
<td></td>
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<td>Fungi / Mites / Nematodes (5)</td>
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<td></td>
<td>Robustocheles mucronata</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Litter/B</td>
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<td>Fungi / Mites (6)</td>
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<tr>
<td></td>
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<td>Fungi / Mites (5,7)</td>
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<td></td>
<td>Stratiolaelaps scimitus</td>
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<td></td>
<td>Proctolaelaps pygmaeus</td>
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<tr>
<td>Total (N)</td>
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<tr>
<td>Moss/D</td>
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</tr>
<tr>
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<tr>
<td>Flowers/E</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Ameroseius pseudoplumosus</td>
<td>5.6</td>
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<tr>
<td>Total (N)</td>
<td>18</td>
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</tr>
</tbody>
</table>


b Relative abundance (%) calculated as the proportion of each species in each sample, based on the total (N) given for each sample.

lower in the treatment with alternative prey than in the control treatments (Figure 3.1d; contrasts after LME). The proportion did not vary significantly through time (LME: \( \chi^2 = 1.54, \text{d.f.} = 2, P = 0.21 \); Figure 3.1d) and there was no significant effect of the interaction of treatment and time on flower damage (LME: \( \chi^2 = 2.38, \text{d.f.} = 2, P = 0.30 \)).

In week 2, we observed low numbers of the astigmatic mite *Tyrophagus putrescentiae* in all treatments, this mite can also serve as alternative prey for predators. After 8 weeks, we found unidentified predatory mites in the control (without predators), but these were not encountered in the tenth week. From the samples of predatory mites taken in the last (tenth) week (maximum 10 individuals), we found mainly *Cosmolaelaps* n. sp. and *P. pygmaeus* in the treatment with alternative food (average ± s.e.: 6 ± 1.15 and 3 ± 0.58, respectively) and in the treatment without alternative food (1.33 ± 0.33 and 0.66 ± 0.66). The numbers of other predatory mite species were lower; these were not identified.

**Thrips control by *Cosmolaelaps* n. sp.**

The results with *Cosmolaelaps* n. sp. as the only predator were comparable to those described in the previous experiment. The effect of treatment on the average numbers of thrips per leaf varied significantly through time (Figure 3.2a; LME: interaction of treatment with time: \( \chi^2 = 13.3, \text{d.f.} = 2, P<0.0013 \)). This interaction occurred because the densities of thrips remained low in the treatment with alternative food but increased in the other treatments, which differed significantly from the former (Figure 3.2a). The effect of treatments on the average numbers of thrips in the flow-

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**Figure 3.1.** Effect of addition of *Carpoglyphus lactis* as alternative prey for litter-inhabiting predators on (a) the average numbers of thrips on leaves (± s.e.); (b) the average numbers of thrips in the flowers (± s.e.); (c) the average proportion of damaged leaves (± s.e.) and (d) the average proportion of damaged flowers per week (± s.e.). Predators were released in the first week, thrips were released in the second week, leaf sampling started in the third week and flower sampling in the fourth week. Different letters indicate significant differences among treatments (contrasts with general linear hypothesis testing after LME, P<0.05).

**Figure 3.2.** Effect of *Cosmolaelaps* n. sp. with or without alternative prey (*Carpoglyphus lactis*) on (a) the average (± s.e.) number of thrips on leaves; (b) the average numbers of thrips in the flowers (± s.e.); (c) the average proportion of damaged leaves (± s.e.); (d) the average proportion of damaged flowers per week (± s.e.) and (e) the average number of *Cosmolaelaps* n. sp. adults in litter samples (± s.e.). Predators were released in the first week, thrips were released in the second week, leaf sampling started in the third week and flower sampling in the fourth week. Different letters indicate significant differences among treatments (contrasts with general linear hypothesis testing after LME, P<0.05).
ers varied significantly through time (Figure 3.2b; LME: interaction of treatment with time: $\chi^2 = 7.4$, d.f. = 2, $P<0.02$). This interaction occurred because the densities of thrips remained low in the treatments with Cosmolaelaps n. sp, but increased significantly in the treatment without predators (Figure 3.2b).

The proportions of damaged leaves varied differently through time among treatments (Figure 3.2c; LME: interaction of treatment with time: $\chi^2 = 19.9$, d.f. = 2, $P<0.0001$). It was significantly lower in the treatment with alternative food than in the two other treatments (Figure 3.2c). There was also a significant effect of treatments on flower damage (Figure 3.2d; LME: $\chi^2 = 14.0$, P<0.001). Releasing Cosmolaelaps n. sp. independently of the addition of alternative prey resulted in flowers with less thrips damage. The proportion of damaged flowers varied significantly through time (LME: $\chi^2 = 4.26$, d.f. = 2, $P = 0.039$; Figure 3.2d), and there was no significant effect of the interaction between treatment and time ($\chi^2 = 0.49$, d.f. = 2, $P = 0.78$).

The addition of alternative prey significantly increased the densities of predators (Figure 3.2e; LME: $\chi^2 = 20.5$, d.f. = 1, $P<0.0001$), which did not vary significantly through time ($\chi^2 = 1.57$, d.f. = 1, $P = 0.21$) and there was no significant effect of the interaction of treatment with time ($\chi^2 = 0.74$, d.f. = 1, $P = 0.39$). We did not find other species of predators in these samples. We found C. lactis in all replicates in which it was released.

**Discussion**

Rose is a prime example of an ornamental that must be damage-free to be marketable (Parrella et al. 1999). Introducing generalist predators has been a successful method of biological control (Symondson et al. 2002), mainly in vegetable crops. One way of improving biological control to meet the standards for ornamentals is to boost the densities of predators in the crop before pest invasion. Nowadays, growers add food to above-ground plant parts to increase the densities of various generalist predators (Vangansbeke et al. 2014). Studies have shown that adding food or prey can enhance biological control (van Rijn et al. 2002; Nomikou et al. 2002, 2010; Janssen & Sabelis 2015; Kumar et al. 2015). These studies, however, concerned above-ground food webs. Only few studies show that biological control can be improved by linking food webs inhabiting the litter to above-ground food webs (Muñoz-Cárdenas et al. 2017). Here, we show that adding food for predators in the litter reduces pest damage on above-ground plant parts.

We found different species of predators in the litter samples collected in commercial greenhouses after adding alternative prey (Table 3.1). The dominating species was Cosmolaelaps n. sp. (Table 3.1), from a genus that is known to occur in litter of natural forests (Furtado & de Moraes 2015). Another species that was abundant in four of the five samples was *P. pygmaeus* (Table 3.1), which has been reported from soil in general and in association with plants, mammals, bark beetles and their galleries, birds, insects, rotting fruits, stored products and bird and mammal nests (de Moraes et al. 2015). It is possible that the different predatory mites in the litter of the greenhouse came from the soil or were carried by insects invading the crop. In the litter, these predatory mites might feed on other arthropods and fungi that are commonly associated with the decomposition of litter (Walter & Proctor 2013). We found that both species fed on the alternative prey *C. lactis* but only Cosmolaelaps n. sp. fed on western flower thrips. This coincides with findings of other authors (de Moraes et al. 2015).

We investigated the effect of *Cosmolaelaps* n. sp plus alternative food on thrips populations because we previously observed that these predators benefit from the alternative prey *C. lactis* and we suspected that they might play a role in thrips control (Muñoz-Cárdenas et al. 2017). Another reason for investigating *Cosmolaelaps* n. sp. is that plants that were left in the greenhouse outside cages and that harbored *Cosmolaelaps* n. sp. predators were less likely to become contaminated by thrips (K. Muñoz-Cárdenas, pers. obs.).

The various predator species present in the litter in the experiment ‘Thrips control by litter-inhabiting predators’ could have engaged in negative predator-predator interactions such as competition or intraguild predation, which could have affected biological control (Rosenheim et al. 1995; Messelink & van Holstein-Saj 2011; but see Janssen et al. 2006). However, thrips densities on leaves were lower when multiple predators plus alternative prey were present than in the controls without predators or without alternative food (Figure 3.1). As was found here, several studies show that the effect of the presence of multiple predators on pest control can be neutral or positive (Gillespie & Quiring 1990; Losey & Denno 1998; Sokol-Hessner & Schmitz 2002; Wiedenhoff et al. 2004; Thoeming & Poehling 2006; Snyder et al. 2008; Wu et al. 2016); however, none of these publications used alternative food. Yet, other studies show negative effects of the presence of multiple predators on pest control (Rosenheim et al. 1993, 2004; Rosenheim 2001; Schausberger & Walzer 2001). In another study, we also studied the effect of multiple predators on pest control, showing that releasing the predatory mites *A. swirskii* on the plants and *M. robustulus* in the litter, each with their own alternative food, also resulted in a positive or neutral effect on thrips control (Chapter 4).

Because the experiments of population dynamics of thrips with several predator species and with *Cosmolaelaps* n. sp alone were carried out at different times for logistical reasons, assessing the effect of single or multiple predators on thrips control is not straightforward. To nevertheless compare thrips control with multiple and single predator species, we calculated the proportional decrease of thrips densities by predators with alternative food relative to the treatment without predators for both
experiments. Thus, we compared two time series with relative changes of the thrips populations. The two time series did not differ significantly for thrips found in the flowers (glm with Gaussian error distribution: $F_{1.8} = 0.007, P = 0.93$) of for the thrips on leaves ($F_{1.4} = 0.79, P = 0.38$), suggesting that Cosmolaelaps n. sp. alone was as efficient in reducing thrips densities as it was together with the other predator species. Further research is needed to assess the effects of interactions among soil predators on biological control.

In both experiments reported here, thrips densities decreased when adding food to a single predator or to a community of predators in the litter. Leaf damage was also reduced compared with the control and a treatment without alternative prey (Figure 3.2). We did not observe short-term increases of thrips densities (as in apparent mutualism), perhaps because predators were released before the thrips. In another study, where thrips were released before the predators, we found a short-term negative effect when adding alternative food for thrips, as in apparent mutualism (Chapter 4).

In the current experiment, there were high densities of thrips in the flowers causing damage in all treatments with Cosmolaelaps n. sp., and this is not acceptable for commercial growers. Hence, further studies are needed to investigate the possibilities of optimizing control methods with this predator species. Moreover, it is worthwhile to investigate the potential of adding alternative food for Cosmolaelaps n. sp. to control thrips in crops with higher damage tolerance, but also for the control of other pests with stages living in the soil. It is estimated that around 75% of all insect pests inhabit the litter or soil at some stage of their lives (Wahab 2010) and having high densities of Cosmolaelaps n. sp. as a result of adding alternative prey could increase pest control.

Another avenue to increase control of thrips in roses would be to combine Cosmolaelaps n. sp. with canopy-dwelling predators plus alternative food. In the case of the plant-dwelling predator A. swirski, there can be negative interactions with Cosmolaelaps n. sp., such as competition or intraguild predation that could affect thrips biological control. This topic deserves further study; however, we expect that the simultaneous use of these two species would not affect thrips control based on a study we performed in which we released A. swirski together with another species of litter-inhabiting predator (Chapter 4).

Summarizing, we showed that adding alternative food in the litter increased the control of an important pest species by litter-inhabiting predators. Most studies of food webs associated with plants focus on above-ground and below-ground communities that are linked via the plant (Scheu 2001), for example, how below-ground herbivores or mycorrhiza modify plant quality, thus affecting the performance of above-ground herbivores (Masters et al. 1993; Gange & West 1994; Brussaard 1998). Mortimer et al. 1999; A’Bear et al. 2014). Less attention has been given to herbivores with stages that feed on above-ground plant parts and other stages that occur below-ground (Johnson et al. 2016). Here, we show that such herbivores can form a direct link between above-ground and below-ground food webs. We suggest that adding alternative prey for litter-inhabiting predators could also increase biological control of other pests that inhabit the litter or soil at some stage of their lives, also in other crops.

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CHAPTER 3 | ALTERNATIVE PREY FOR PREDATORS IN LITTER REDUCES CANOPY DAMAGE


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CHAPTER 3 | ALTERNATIVE PREY FOR PREDATORS IN LITTER REDUCES CANOPY DAMAGE
Single and combined predator releases with alternative food increases thrips control in an ornamental crop

Karen Muñoz-Cárdenas, Ada Leman, Marcus V. A. Duarte, Gerben J. Messelink & Arne Janssen

Many pest species spend part of their life cycle in the soil or litter and another part on the above-ground plant parts. Therefore, a viable strategy to control such pests may be to release a combination of canopy-dwelling predators and litter-inhabiting predators. However, when predators attack the same pest population, there will be competition at some point, even if they attack different stages and different parts of the population. This competition may be reduced when predators have alternative food sources. The effect of adding alternative food for predators can have positive or negative effects on pest control, depending on the time frame and fluctuations of prey and predator populations. We tested the effect of single or combined predator releases with alternative food on thrips control on cut roses in an experimental greenhouse. In a first experiment, we released thrips before the predators *Amblyseius swirski* and the soil-dwelling predatory mites *Macrocheles robustulus* and *Stratiolaelps scimitus*, using *Acarus siro* or pollen as alternative food. In a second experiment, we released *A. swirski* and *M. robustulus* before the thrips and supplied *Typha* spp. pollen on the plants for *A. swirski* and *A. siro* in the litter for *M. robustulus*. We found best thrips control when *A. swirski* plus pollen were released, independently of the release of soil-dwelling predatory mites. Furthermore, better thrips control was achieved when predators were released before the thrips. Our results suggest that soil-dwelling predatory mites, which are present in the litter layer in many commercial greenhouses, do not affect thrips control by *A. swirski*. Hence, growers can use this latter species independent of the presence of these soil-dwelling predators. Growers should preferably release *A. swirski* plus pollen before thrips invade the crop.
CHAPTER 4 | THrips CONTROL IN CUT ROSES

Introduction

Prey are often attacked by several species of predators (Polis 1991; Polis & Strong 1996; Sih et al. 1998). Different species of natural enemies can have additive effects on prey populations, meaning that their combined effect equals the sum of the effects of the different natural enemy species (Sih et al. 1998; Casula et al. 2006). Multiple natural enemies can also cause further increases in prey densities, for example because of the occurrence of interactions such as intraguild predation among predators (Ferguson & Stiling 1996; Rosenheim et al. 1993, 1994; Roseboom 2016; Snyder & Ives 2001), or because the response of the prey to a species of natural enemy decreases the risk of being attacked by the other species (Magalhães et al. 2002; Sih et al. 1998). Furthermore, the combined effect of several species of natural enemies can cause further decreases in prey densities than their additive effect, for example because they feed on different stages of the prey (Takagi & Hirose 1994), or because the response of prey to one natural enemy species makes them more vulnerable to the other species (Losey & Denno 1998, 1999).

In biological control, the effect of multiple natural enemies versus the effect of one natural enemy varies according to the crop and the species involved (Chang 1996; Ferguson & Stiling 1996; Sih et al. 1998). There are examples of disruption of biological control by multiple natural enemies (Finke & Denno 2004), of neutral effects, where the addition of other species of natural enemies does not result in further reduction of pest populations (Cardinale et al. 2003), and positive effects, which can be additive or synergistic, increasing pest control (Losey & Denno 1998). Studies showing interactions among predators that have a positive effect on pest control involve systems in which predators attack the pest at different locations or on different plant parts, different seasons, or at different life stages (Murdoch et al. 1984; Onzo et al. 2004). However, when predators attack the same pest population, they will compete in the end, even when they attack different stages or different parts of the population. Such competition may be reduced when the predators have alternative food (Onzo et al. 2005). Here, we investigated the effect of adding alternative food for a combination of predator species that attack different stages of a pest and at different locations (i.e. the canopy and the litter).

The effects of adding alternative food, such as pollen, to increase biological control by generalist predators has been widely investigated (McMurtry & Scriven 1966; van Rijn et al. 2002; Nomikou et al. 2002, 2010). There can be positive effects on biological control of adding alternative food, because the addition of food results in an increase in predator densities in the long term, after one or a few predator generations. These higher predator densities result in more attacks on pest individuals (apparent competition; Holt 1977), enhancing biological control. However, during the first few generations of the predator, biological control might also be negatively affected by adding alternative food, because predators may feed on the alternative prey instead of feeding on the target pest, or because adding alternative food results in predator satiation (apparent mutualism; Holt 1977; Abrams & Matsuda 1996).

Several studies have demonstrated that adding pollen as food for predators to the plant canopy can indeed increase pest control (McMurtry & Scriven 1966; Kennett et al. 1979; van Rijn et al. 2002; Maoz et al. 2009; Nomikou et al. 2010; Azad et al. 2014; Leman & Messelink 2015; Duarte et al. 2015). Alternative prey for predators can also be added to the litter. In previous studies, we showed that litter-inhabiting predators that feed on litter-inhabiting stages of an important plant pest (western flower thrips) can benefit from alternative prey supplied in the litter, resulting in increased thrips control (Muñoz-Cárdenas et al. 2017; Chapter 3). So far, studies focusing on releases of combinations of predators on plants and in the litter or soil have not addressed the effect of alternative food sources for both predators on biological control. Thus, our question is whether a combination of predators on the plants and in the litter, each with their own alternative food, would increase biological control.

We studied the control of Western flower thrips (Frankliniella occidentalis) in greenhouse cut roses. In the particular case of this pest, adding pollen as alternative food can be risky, because pollen is also a high-quality food source for thrips (van Rijn et al. 2002; Chitturi et al. 2006; Leman & Messelink 2015; Vangansbeke et al. 2016). There are only few studies on the effect of adding pollen for predatory mites on thrips control in ornamentals (Delisle et al. 2015; Leman & Messelink 2015). In one of these studies, potted chrysanthemum plants received more damage when the predatory mite Amblyseius swirskii (Athias-Henriot) (Acarac: Phytoseiidae) was released together with pollen than without pollen (Delisle et al. 2015). However, this experiment lasted for 4 weeks (Delisle et al. 2015), so the evaluation period may have been too short to observe the long term positive effect of adding pollen for predators on thrips control. The study of Leman & Messelink (2015) on chrysanthemum lasted for 8 weeks and shows enhanced control of thrips when predatory mites are provided with pollen, but plant damage was not assessed. Another study, in rose plants, shows increases in densities of A. swirskii and other phytoseid predators when adding pollen, but thrips densities and plant damage were not assessed (Pijnakker et al. 2016). Because thrips spend part of their life cycle in the litter or soil, attempts have been made to increase control of thrips by using canopy-dwelling predators that attack thrips larvae together with soil-inhabiting predators that attack prepupae and pupae (Wietbroft et al. 2004; Thoeming & Poehling 2006; Manners et al. 2013; Pozzebon et al. 2015). These studies did not find a significant increase in thrips control using such combinations of predators. However, they did not supply alternative food for the predators. We therefore conducted two experiments. In the first experiment, we supplied one type of alternative prey in the litter for a combination of predators. Previous studies
(Muñoz-Cárdenas et al. 2017; Chapter 3) show that both the soil-inhabiting and the canopy-dwelling predators do use this alternative food when supplied to the litter. Here, we assessed the combined effects of these predators with alternative food on thrips control. In the second experiment, we supplied each predator with its own alternative food; alternative prey was again added to the litter, but we also added pollen to the above-ground plant parts.

**Materials and methods**

**The experimental system**

Plants were grown in rockwool (Grodan®) and nutrients were applied in the irrigation system. Rose plants in rockwool slabs were placed on top of gutters (JBHydroponics®) at around 60 cm from the ground. In the Netherlands, cut rose plants are grown in this way for 5-10 years, resulting in the accumulation of litter on the greenhouse floor. Some plant branches touch this litter layer, forming a connection between the above-ground and below-ground habitats. In this study we simulated these conditions in an experimental greenhouse under conditions resembling those of commercial crop production systems.

The western flower thrips *Frankliniella occidentalis* (Pergande) causes important economic damage in many different crops, including roses (Park et al. 2002; Manners et al. 2013). The predators used in this study were *A. swirskii*, which is commonly released to control thrips and whiteflies in various crops (Nomikou et al. 2001, 2002; Messelink et al. 2008), and the soil-dwelling predatory mites *Macrocheles robustulus* (Berlese) and *Stratiolaelaps scimitus* (Womersley), which can also control thrips (Berndt et al. 2004; Messelink & van Holstein-Saj 2011) and sciarids (Grosman et al. 2011). We used *Typha latifolia* L. or *Typha angustifolia* L. pollen as alternative food for *A. swirskii*. Both predators feed on the alternative prey *Acarus siro* (L.), which was released in the litter.

**Arthropod cultures and pollens**

Western flower thrips (*F. occidentalis*) were reared on flowering chrysanthemum plants (*Dendranthema grandiflora* Tzeli, var. Miramar) in a greenhouse compartment. The flour mite *A. siro* was reared on wheat bran (Ramakers & van Lieburg 1982). *Amblyseius swirskii* (Swirski-mite®), *S. scimitus* (Entomite M®) and *M. robustulus* (Macro-mite®) were obtained from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands). For the first experiment, we used *T. latifolia* pollen, collected from plants in the surroundings of the experimental station (Bleiswijk, The Netherlands). It was dried and stored at -20 °C. For the second experiment, we used *T. angustifolia* pollen (Nutrimite®) provided by Biobest (Westerlo, Belgium).

**Study area and plant material**

The experiments were performed in a greenhouse compartment at Wageningen UR Greenhouse Horticulture in Bleiswijk, The Netherlands. The compartment was 96 m², the average temperature was 22.2 °C, RH 80%, photoperiod L13:D11. Artificial light was 10,000 lux, and was switched on when natural light in the greenhouse was less than 150 Watt/m². The windows of the greenhouse were provided with insect gauze (0.40 × 0.45 mm) to prevent arthropod invasions. Twenty cages, consisting of a plastic frame (1 × 1.5 × 2 m) covered with insect-proof mesh (mesh size 0.22 × 0.31 mm), were placed inside this compartment. Cages were located about 50 cm from each other. Each cage had two zippers at the front for easy access. In March 2014, 10 rose plants (1 year old, var. Avalanche +++; Leo Ammerlaan, Bleiswijk) rooted in rock wool were placed in each cage on top of two gutters (JBHydroponics®) (5 plants/gutter). The plant density (6 plants/m²) is characteristic of Dutch commercial roses. Litter from a commercial greenhouse (Royal Roses, Stompwijk, The Netherlands) was sterilized (120 °C, 20 min) and placed below the gutters of the cages. To start the experiment with plants that were free of pests and diseases, we treated all plants prior to the experiment with a mix of pyridylethylamide (Luna privilage, Bayer) against powdery mildew and lufenuron (Match, Syngenta) and pyridalyl (Nocturn, Nufarm) against western flower thrips. To prevent powdery mildew infection, we used a Sulphur evaporator until the week before the start of the experiment. Because the cages had no bottom, we built a barrier of sand (10 cm wide, 10 cm high) on the floor against the cage walls on the inside and outside of all the cages to prevent escapes of litter-inhabiting mites. During both experiments, a standard nutrient solution was provided to the plants with a drip irrigation system. Roses were harvested once per week, stems were bent and buds from these stems were removed according to commercial practice. As a preventive measurement, each cage was weekly supplied with *Encarsia formosa* Gahan (150 individuals/cage, En-strip®, Koppert) against whiteflies (*Trialeurodes vaporariorum* Westwood) and *Phytoseiulus persimilis* Athias-Henriot (200 individuals/cage, Spidex®, Koppert) against spider mites (*Tetranychus urticae* Koch).

**Experiments**

In previous studies (Muñoz-Cárdenas et al. 2017; Chapter 3) we found that the positive effect of adding alternative prey on biological control of thrips in roses is achieved only when the predator populations are established before pest invasion (also known as the predator-in-first approach; Ramakers 1990; Kutuk & Yigit 2011; Kumar et al. 2015). However, in the case of ornamentals, there are no studies comparing the effect of predator pre-establishment with releases of predators after pest invasions on biological control. For this reason, we carried out the first experiment by releasing predators with alternative food after thrips. For the second experiment,
predator populations were allowed to establish by releasing them with their alternative food before releasing the pest.

**Thrips control with two predators and one alternative food source**

This experiment was carried out for 19 weeks from June to October of 2014. Frankliniella occidentalis adults were released during the first 3 weeks of the experiment to simulate a thrips invasion (see Table 4.1 for numbers). Thrips adults were collected from the colony in 10 ml pipette points with a vacuum pump. The tips were closed with cotton wool and transferred to the greenhouse for release of thrips inside the cages. In the fifth week, the cages received one of five treatments, each with four replicates (cages): 1) no predators, 2) A. swirskii, 3) A. swirskii + pollen, 4) M. robustulus + S. scimitus + A. siro, or 5) M. robustulus + S. scimitus + A. siro + A. swirskii. Females of A. swirskii were taken from the bottles of the commercial product using a fine brush and placed on sweet pepper leaf discs (Capsicum annuum L.). Subsequently the discs were placed on the rose plants in the cages. These predators were released in week 5 and week 12 (Table 4.1). As alternative food source for A. swirskii, T. latifolia pollen (1 g/cage) was evenly distributed with a fine brush on the plants in the cages according to the treatment (Table 4.1).

Food and substrate for the alternative prey A. siro consisted of sterilized wheat bran, coarse vermiculite, peat, water and yeast. Subsequently A. siro were added to this mix. Individuals of the soil-dwelling predatory mites S. scimitus and M. robustulus were added to this mix of food and alternative prey. The release of A. siro as alternative prey was repeated in the twelfth week (Table 4.1).

Cages were sampled every 2 weeks (from weeks 7 to 19), by haphazardly collecting twenty leaves from the top and lower parts of the plants per cage. The thrips (larvae and adults) and predatory mites (larvae, nymphs and adults) were counted on each leaf under a stereo-microscope in the laboratory and the predatory mites were mounted on slides for identification. The number of damaged leaves per cage was also recorded.

For sampling litter-inhabiting predators, we took 250 ml of litter from each cage two times during the experiment (weeks 10 and 17) and incubated it in Tulip grfen funnels during 1 week. Subsequently, the samples were sieved, predatory mites and alternative prey were counted and the predatory mites found were mounted on slides for identification. Mature flowers were also sampled; five open flowers per cage were collected in plastic bags every week (from weeks 9 to 19), and the presence of scars on the petals and on the sepal of the flowers was recorded. Flowers were considered as damaged if they had any deformations or scars on the petals. Flowers were subsequently washed with 70% alcohol on a mesh (100 μm) and the thrips and predators remaining on the mesh were counted under a stereo-microscope.

Average numbers of thrips per flower and per leaf were log(x+1) transformed, the average numbers of thrips per leaf and the average number of A. swirskii per leaf and per flower were log(x<0.01) transformed. Numbers of M. robustulus and S. scimitus were log(x+1) transformed before analysis. Because almost no A. swirskii were encountered in the flowers during the first 14 weeks of the experiment, only data from week 15 onwards were evaluated for this species. All data were analyzed with linear mixed effects models (LME of the nlme package of R, Pinheiro et al. 2014) with experimental unit (cage) as random factor and time and treatment and their interaction as fixed factors. Contrasts among treatments were assessed with the glht function with Tukey HSD (package lsmeans of R; Lenth 2016). We found A. siro individuals in the litter only in week 17, not in week 10. The numbers per sample of these mites were therefore compared using generalized lineal models (GLM instead of LME) with treatment as factor and a quasi-Poisson error distribution. All statistical analyses were done using R (R Development Core Team 2015). Leaf sampling started in week 7, flower sampling in week 9 when the first flowers could be harvested.

**Thrips control with two predators and two alternative food sources**

This experiment was performed using the same cages with the same plants as in the previous experiment. To remove arthropods and mildew, we sprayed with a solution containing the insecticides fioncanid (Teppeki®, Belchim) and abamectin (Vertimec gold®, Syngenta), the fungicide dodemorf (Meltatox®, BASF) against powdery mildew, and heptamethyltrisiloxane (Silwet gold®, Certis Europe) as wetting agent. Furthermore, the plants were pruned, the stems that had flowered were bent, all colored buds were removed, and the litter from the bottom of the cages was removed for sterilization and placed back under the gutters in the cages. After allowing the plants to regrow, an experiment was done from April to July of 2015. There were five treatments with four repetitions (cages): 1) untreated (no predators), 2) M. robustulus + A. siro, 3) A. swirskii + pollen weekly, 4) M. robustulus + A. siro + A. swirskii + pollen weekly, or 5) A. swirskii + pollen (interrupted). We released only M. robustulus as litter predators in this experiment because they were the dominant species in the previous experiment. To test whether the frequency of adding pollen had an effect on thrips densities, pollen was added weekly in two of the treatments, and in one other treatment, the pollen supply was interrupted (Table 4.2). Thrips, alternative prey and predators were released as in the previous experiment, timing of their release can be found in Table 4.2. Leaves, flowers and litter were sampled as in the previous experiment. For data analysis, average numbers of thrips per flower were log(x+1) transformed, thrips per leaf were log(x<0.01) transformed. The densities of A. swirskii in flowers were very low during the first 9 weeks of the experiment; these data were therefore excluded from the analysis. The remaining densities were log(x<0.1) trans-
formed, as were the densities of this predator per leaf. Densities of *M. robustulus* and *A. siro* were log(x+1) transformed. Proportions of damaged leaves and flowers were arcsin √x transformed. All transformed data were compared using LME as in the previous experiment.

**Results**

**Thrips control with combined predators and one alternative food source**

The average numbers of thrips per flower differed significantly among treatments through time (Figure 4.1a; LME, interaction of treatment with time: d.f. = 4, \( \chi^2 = 74.5, P < 0.0001 \)). This was because the average thrips densities increased in most treatments, but decreased in the treatment with *A. swirskii* plus pollen. Thrips densities were on average highest in the control and with *A. swirskii* without pollen, and lowest with *A. swirskii* plus pollen (Figure 4.1a). Overall, thrips densities in the flowers were high, with unsatisfactory control, except in the treatment with *A. swirskii* plus pollen during the last 3 weeks of the experiment (Figure 4.1a; contrasts with glht function of package lsmeans).

The average numbers of thrips per leaf also differed significantly among treatments through time (Figure 4.1b; LME, interaction of treatment with time: d.f. = 4, \( \chi^2 = 17.1, P = 0.0018 \)). This may have been because the densities of thrips dropped in the treatment with *A. swirskii* plus pollen during the last few weeks, as it did in the flowers. However, none of the contrasts among treatments was significant (Figure 4.1b; contrasts).

*Amblyseius swirskii* was practically absent in the flowers during the first 14 weeks of the experiment (Figure 4.1c). Analysis of the data from week 15 onwards showed a significant difference among treatments through time (LME: d.f. = 2, \( \chi^2 = 6.91, P = 0.032 \)), caused by the higher numbers of *A. swirskii* in the presence of pollen during the last 4 weeks than in the other treatments (Figure 4.1c). The average density of *A. swirskii* per leaf also differed significantly among treatments through time (Figure 4.1d; LME, interaction: d.f. = 2, \( \chi^2 = 10.8, P = 0.0044 \)), likely caused by the high numbers of predators towards the end of the experiment in the treatment with *A. swirskii* plus pollen, but not in the other treatments (Figure 4.1d; contrasts).

By releasing the thrips before the predators and the alternative food, all the flowers had thrips damage on the petals, independently of the treatment (K. Muñoz-Cárdenas, pers. obs.). Moreover, the proportion of leaves damaged by thrips was high in all treatments (Figure 4.1e), but differed significantly among treatments through time (LME, interaction: d.f. = 4, \( \chi^2 = 13.3, P = 0.01 \)). This was because the proportion of damaged leaves in the treatment with *A. swirskii* plus pollen was the highest at the start of the experiment and lowest at the end of the experiment. Nevertheless, the differences among treatments through time were not significant (Figure 4.1e; contrasts).

![Figure 4.1](image-url)
The numbers of *M. robustulus* in litter samples differed significantly among treatments through time (LME, interaction: d.f. = 1, \( \chi^2 = 9.7, P = 0.0018 \)). This was because the numbers of this predator did not differ between treatments in the tenth week of the experiment, whereas the numbers were significantly higher in the treatment without *A. swirskii* at 17 weeks (FIGURE 4.2a). The densities of *S. scimitus* in the litter samples did not differ significantly among treatments through time (LME, interaction: d.f. = 1, \( \chi^2 = 0.0036, P = 0.95 \)). There were significantly more *S. scimitus* present in the litter after 17 weeks than after 10 weeks (FIGURE 4.2b; LME: d.f. = 1, \( \chi^2 = 10.8, P = 0.001 \)). Besides the species of litter predators released, we found high densities of *Lasioseius fimentorum* (Acari: Podocinidae) (average ± s.e. = 68.1 ± 37.3) in the treatments with soil dwelling predators in week 10. Probably they came from the soil in the greenhouse and their populations increased as a result of the alternative prey added (*A. siro*). After 17 weeks, we found high densities of *Ameroseius* sp. (*Acari: Ameroseiidae*) (112.3 ± 54.9) in the treatments without releases of soil dwelling predators. At the same time, we found low numbers of *Neoseiulus barkeri* (*Acari: Phytoseiidae*) (6 ± 3.5) and *Geolaelaps aculeifer* (*Acari: Laelapidae*) (5.3 ± 4) in the litter samples of all treatments.

*Acarus siro*, the alternative prey added to the litter, was not encountered after 10 weeks and was therefore added another time in week 12 (TABLE 4.1). This resulted in their presence in the litter after 17 weeks, and their numbers did not differ significantly between treatments (*M. robustulus + A. siro*: average ± s.e. = 847.5 ± 465.1, *M. robustulus + A. siro + A. swirskii*: 535 ± 185.7; GLM: d.f. = 1, \( \chi^2 = 0.2, P = 0.95 \)). The densities of *S. scimitus* per litter sample varied significantly among treatments (LME: d.f. = 2, \( \chi^2 = 3.65, P = 0.05 \)), and there was no significant effect of time or the interaction of treatment with time (d.f. = 2, \( \chi^2 = 0.87, P = 0.35 \)). Densities of *A. siro* on leaves varied significantly among treatments (LME: d.f. = 2, \( \chi^2 = 8.61, P = 0.072 \)). There was a significant effect of treatments with time (d.f. = 2, \( \chi^2 = 0.87, P = 0.35 \)) and there was no significant interaction of treatment with time (d.f. = 2, \( \chi^2 = 3.65, P = 0.16 \)). Densities of *A. swirskii* on leaves were much lower when pollen supply was interrupted (FIGURE 4.3d).

The average numbers of thrips per flower differed significantly among treatments through time (FIGURE 4.3a; LME, interaction: d.f. = 4, \( \chi^2 = 16.1, P = 0.0029 \)). Whereas thrips densities did not differ during the first few weeks, they subsequently increased in the treatments without *A. swirskii* plus pollen, whereas they remained low in all treatments with *A. swirskii* plus pollen (FIGURE 4.3a).

Densities of thrips per leaf did not vary significantly through time (FIGURE 4.3b; LME: d.f. = 4, \( \chi^2 = 0.35, P = 0.56 \)), nor was there a significant interaction of treatment with time (d.f. = 4, \( \chi^2 = 0.87, P = 0.35 \)). There was no significant effect of time (d.f. = 4, \( \chi^2 = 8.61, P = 0.072 \)). There was a significant effect of treatments with time (d.f. = 4, \( \chi^2 = 3.65, P = 0.16 \)), but contrasts among treatments did not reveal any significant differences (FIGURE 4.3b).

The densities of *A. swirskii* per flower were very low during the first 9 weeks of the experiment (FIGURE 4.3c), and did not differ significantly among treatments from week 10 onwards (LME: d.f. = 2, \( \chi^2 = 1.32, P = 0.52 \)). There was no significant effect of time (d.f. = 2, \( \chi^2 = 0.87, P = 0.35 \)) or of the interaction of treatment with time (d.f. = 2, \( \chi^2 = 1.97, P = 0.37 \)). Densities of *A. swirskii* on the leaves varied significantly among treatments (FIGURE 4.3d; LME: d.f. = 2, \( \chi^2 = 25.5, P<0.0001 \)), and there was no significant effect of time (d.f. = 2, \( \chi^2 = 0.045, P = 0.83 \)) or of the interaction of treatment with time (d.f. = 2, \( \chi^2 = 3.65, P = 0.16 \)). Densities of *A. swirskii* on leaves were much lower when pollen supply was interrupted (FIGURE 4.3d).

The proportion of damaged flowers varied significantly through time among treatments (FIGURE 4.3e; LME, interaction of treatment with time: d.f. = 4, \( \chi^2 = 40.1, P<0.0001 \)). Initially, there were few damaged flowers in all treatments, but this subsequently increased in the treatments without *A. swirskii* plus pollen (FIGURE 4.3e).

The average proportion of damaged leaves varied significantly among treatments.
Effect of addition two predators and two alternative food sources on thrips densities, predator densities

A. swirskii

proportion of damaged leaves, (A. swirskii/leaf, (thrips/flower, Thrips control with two predators and two alternative food sources. The timing of releases of the western flower thrips Frankhina occidentalis, predatory mites (Amblyseius swirskii and Macrocheles robustulus) and alternative prey (Acarus siro) or food (pollen) on rose plants. Pollen was either added weekly or interrupted.

Table 4.2. Thrips control with two predators and two alternative food sources. The timing of releases of the western flower thrips Franklinia occidentalis, predatory mites (Amblyseius swirskii and Macrocheles robustulus) and alternative prey (Acarus siro) or food (pollen) on rose plants. Pollen was either added weekly or interrupted.

<table>
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<td>Pollen (interrupted)</td>
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Food in the litter | 1.75 |

a Number of adults released per week per cage.

b Grams added per cage

c Number of immatures and adults.

d Liters of food and substrate for alternative prey containing 1 L of sterilized wheat bran + 200 ml of coarse vermiculite + 300 ml of peat + 250 ml of water + 25 g of baker’s yeast.

There was no statistically significant effect of the treatments on the numbers of M. robustulus in the litter (Figure 4.4; LME: d.f. = 1, \( \chi^2 = 0.10, P = 0.75 \)), the numbers varied significantly with time (d.f. = 1, \( \chi^2 = 4.20, P = 0.041 \)), but the interaction through time (Figure 4.3f; LME, interaction: d.f. = 4, \( \chi^2 = 30.8, P<0.0001 \)); the proportion of damaged leaves was initially low in all treatments, but increased considerably in the treatments without A. swirskii plus pollen (Figure 4.3f). It is noteworthy that the proportion of damaged leaves increased considerably in the last week when the addition of pollen to A. swirskii was interrupted (Figure 4.3f).

Table 4.2. Thrips control with two predators and two alternative food sources. The timing of releases of the western flower thrips Franklinia occidentalis, predatory mites (Amblyseius swirskii and Macrocheles robustulus) and alternative prey (Acarus siro) or food (pollen) on rose plants. Pollen was either added weekly or interrupted.

Figure 4.3. Effect of addition two predators and two alternative food sources on thrips densities, predator densities and proportions of damaged rose leaves and flowers. Predators plus alternative food were allowed to establish before thrips releases (Table 4.2). Treatments were: 1) No predators; 2) Macrocheles robustulus + Acarus siro in the litter; 3) Amblyseius swirskii + pollen weekly applied on leaves; 4) M. robustulus + A. siro + A. swirskii + pollen weekly; or 5) A. swirskii + pollen (interrupted); pollen was added four times during the experiment (Table 4.2). Predators and alternative food were released before thrips (Table 4.2). Shown are average numbers (s.e.) of (a) thrips/flower, (b) thrips/leaf, (c) A. swirskii/flower, (d) A. swirskii/leaf, (e) proportion of damaged flowers, and (f) proportion of damaged leaves. Different letters in the legends represent significant differences among treatments through time.

Figure 4.4. Average numbers (s.e.) of mites found in 250 ml of litter samplings in the treatments in which Macrocheles robustulus was released with the alternative prey Acarus siro.
between treatment and time was not significant (d.f. = 1, $\chi^2 = 0.92$, $P = 0.34$). The densities of the alternative prey A. siro did not differ between treatments (LME: d.f. = 1, $\chi^2 = 0.25$, $P = 0.61$), but varied significantly with time (d.f. = 1, $\chi^2 = 35.8$, $P < 0.0001$; interaction treatment and time: d.f. = 1, $\chi^2 = 0.84$, $P = 0.36$). The averages of A. siro in week 6 were higher than in week 10 (week 6: $M. robustulus + A. siro$: (average ± s.e.) 1787 ± 477; $M. robustulus + A. siro + A. swirskii$ + pollen: 2225 ± 607; week 10: $M. robustulus + A. siro$: 10 ± 6; $M. robustulus + A. siro + A. swirskii$ + pollen: 5 ± 5). Again, we found predatory mites of other families in all treatments (probably Phytoseiidae, Ameroseiidae and Laelapidae) (39.7 ± 21).

Summarizing both experiments, independent of the presence of $M. robustulus$ and A. siro, releasing A. swirskii and adding pollen as alternative food resulted in the largest decrease in thrips densities, the largest increase in A. swirskii densities and the lowest plant damage, compared to the rest of the treatments.

Discussion
Combining predators does not increase or hampers thrips control
In the experiment with combined predators and one type of alternative food, we found lower thrips densities in the flowers of plants with A. siro and M. robustulus in the litter than in the treatment without predators. However, these densities did not differ significantly from those in other treatments with predators (FIGURE 4.1a). At the end of this experiment, we found significantly fewer $M. robustulus$ when it was released in combination with A. swirskii and S. scimitus than when it was released with S. scimitus only (FIGURE 4.2a). This may have been caused by negative predator-predator interactions, such as competition for food or intraguild predation. This topic deserves further study, as well as the role of the other litter-inhabiting mites encountered in biological pest control.

We observed low numbers of S. scimitus when they were released together with $M. robustulus$. Elsewhere (Berndt et al. 2004), this species (synonym Hypoaspis miles) was shown to be an effective predator of thrips in the litter. In their experiments, the plants were confined to small tubes and the thrips mainly pupated in the soil where S. scimitus and M. robustulus were released. In our experiments, thrips had more options for pupation, for example at the base of the plants or in the flowers, thus they may have escaped from predation by these litter-inhabiting predators. This may have caused the low effect of the litter-inhabiting predators on thrips control.

The combined release of the predatory mites A. swirskii with pollen as alternative food on the plant canopy and $M. robustulus$ with A. siro in the litter resulted in increased biological control compared to the release of $M. robustulus$ with A. siro as alternative prey. However, the combined release did not result in better control than releases of only A. swirskii with pollen. Similar effects of combined releases of other species of canopy-dwelling and soil-inhabiting predators on thrips control were found by Wiethoff et al. (2004) and Thoeming & Poehling (2006), but these authors did not study the predators with alternative food. Our results show that single releases of A. swirskii combined with pollen resulted in increased thrips control (FIGURES 4.1 and 4.3). There was no synergistic effect of the two predators (facilitation), as has been found in another system with canopy-dwelling and soil-dwelling predators (Losey & Denno 1998, 1999). However, we also did not find evidence that the soil predator hampered pest control by the canopy-dwelling predator, in contrast to a study in another system (Messelink & van Holstein-Saj 2011).

Many arthropod plant pests spend part of their life cycles in the litter or soil (Wahab 2010). This means that biological control could be directed to the pest in both habitats by releasing predators in the plant canopy and in the litter or soil. In the experiment with two predators and two alternative food sources, we did not find a significant effect of $M. robustulus$ on thrips populations in the flowers, whereas Messelink & van Holstein-Saj (2008) show that this predator can substantially reduce thrips populations. In a previous study (CHAPTER 3), we found that another litter-inhabiting predatory mite, Cosmolaelaps n.sp., can control thrips in rose plants when released with alternative food in the litter at the foot of the plant. In the present study, we released the predators in the litter layer under the gutters on which rose plants were located, thus the predators were not in direct contact with the plants. Again, it is possible that some thrips pupated at the base of the plants, thus escaping from predation by $M. robustulus$. Further studies could therefore include treatments in which litter-inhabiting predators with alternative food are added at the foot of the plant, just below the canopy.

Supplying pollen to Amblyseius swirskii increases thrips control
Our results confirm that adding alternative food for predators can increase biological control even if the pest can also benefit from the alternative food (van Rijn et al. 2002). At the start of the experiment with one type of alternative food, however, we found the largest numbers of thrips in the flowers and a larger proportion of damaged leaves in the presence of A. swirskii and pollen (FIGURE 4.1a,e). This could have been caused by a direct short-term positive effect of pollen on thrips densities, by apparent mutualism (i.e. an indirect short-term positive effect mediated by the predators, Holt 1977), or both.

In the experiment with two types of alternative food, we found that adding pollen on plants weekly or interrupted (4 times in 14 weeks) caused decreases of thrips densities and plant damage (FIGURE 4.3). There were no significant differences in
thrips densities or plant damage between the treatments with weekly or interrupted pollen supply (Figure 4.3e,f), but there were significantly lower numbers of A. swirskii with interrupted pollen supply (Figure 4.3d) and an increase in damaged leaves in this treatment (Figure 4.3f). This suggests that it is not a good idea to interrupt pollen supply when thrps invade the crop. To our knowledge, there are no studies that investigate the most adequate frequency of food supply to predators; further studies should be devoted to this topic. The commercial recommendation for growers is to add pollen to the crop every 2 weeks (Pijnakker et al. 2016), according to our results, growers could add pollen weekly, which results in more predators on the leaves compared with an interrupted pollen supply (Figure 3d).

We found no negative effect of M. robustulus on thrps control by A. swirskii. This suggests that growers can preventively release A. swirskii and add pollen on the plants for control of F. occidentalis in roses even when these predators are in the litter.

Pre-establishment of predators is necessary for thrps control
Because the two experiments reported here were carried out at different times for logistical reasons, assessing the effect of releasing predators before or after releasing thrps is not straightforward. To nevertheless compare thrps control in the two experiments, we calculated the proportional decrease of thrps densities inside flowers, the marketable plant part, and on the leaves. Specifically, we calculated this decrease in the treatments with A. swirskii with pollen relative to the control (without predators and alternative food) for both experiments. This yielded two time series with relative changes of the thrps populations in the flowers, and two time series for the leaves. The average proportional thrps density in the flowers was 0.74 (s.e. 0.18) when thrps were released first and 0.34 (s.e. 0.078) when the predators were released first. Overall, this difference was significant (GLM with Gaussian error distribution: \( F_{1,18} = 6.41, P = 0.021 \)). For the leaves, we did not find a significant difference in thrps densities (GLM with gamma error distribution: \( F_{1,10} = 2.56, P = 0.14 \)). These comparisons suggest that pre-establishment of A. swirskii with pollen results in better control of thrps in rose flowers, confirming the findings of Muñoz-Cárdenas et al. (2017) and the results of other authors in vegetable crops regarding the importance of pre-establishment of predators for thrps control (Ramakers 1990; Kutuk & Yigit 2011; Kumar et al. 2015).

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Biological control of pests is an important component in crop management (Bale et al. 2008). Usually, crops are attacked by various pest species, therefore, generalist predators have been considered as a solution for pest control (Symondson et al. 2002). Contrary to specialist predators, generalist predators can feed on different prey species and on plant-provided foods (Symondson et al. 2002). For a long time, there was scepticism regarding the use of generalists for pest control: their ability to feed on different sources was sometimes considered a disadvantage for biological control because it was interpreted as generalist predators not being well adapted to the pest (Huffaker et al. 1969). However, since the 1950s, numerous studies have shown that the presence of alternative food for generalist predators on the plant did not have negative effects on biological control, but rather improved it (Huffaker & Kennett 1956; Collyer 1964; van Rijn et al. 2002). In theory, two prey populations can affect each other’s densities indirectly when they are attacked by the same population of natural enemies (Holt 1977). The fact that generalist predators feed on different prey or other foods could negatively affect biological control because they might feed on the alternative food or prey, thus releasing the target pest from attacks, this short-term effect is called apparent mutualism (Murdoch 1969; Abrams & Matsuda 1996). This positive effect of adding alternative prey or food on pest densities is obviously detrimental for biological control. In the long-term, however, the densities of prey may decrease in response to increases of predator populations, increasing biological control (Collyer 1984; Karban et al. 1994; Müller & Godfray 1997). Hence, the effect of the alternative food or prey on each pest densities can be positive (apparent mutualism) or negative (apparent competition). When population densities do not reach equilibria, but show cycles, apparent mutualism can occur also in the long-term, resulting in repeated satiation of the shared predators and repeated reduced predation on the other prey, thus affecting biological control negatively (Abrams et al. 1998). Moreover, the use of generalist predators might be also detrimental for biological control because they can compete for prey or food with other natural enemies and they can also engage in negative interactions such as intraguild predation (Rosenheim et al. 1995). However, the use of combinations of species of generalist predators or combinations with other natural enemies do not always result in negative effects on biological control (Cardinale et al. 2003; Losey & Denno 1998). Summarizing, generalist predators can mediate interactions between prey (apparent mutualism, apparent competition; Holt et al. 1977) and can also engage in positive
or negative interactions with other natural enemies. Thus, the outcome of using generalist predators for biological control can best be predicted when studying the different interactions among predators and prey.

Because generalist predators interact with several species, they increase connectivity within and among food webs. In the past, above-ground and below-ground food webs were studied independently. However, it has become clear that these two food webs are connected, which affects the composition and structure of the communities in both habitats (Gange & Brown 1989; van der Putten et al. 2001; Bezemer & van Dam 2005; A'Bear et al. 2014). Generalist predators can form direct links between above-ground and below-ground food webs when they feed on pests present on above-ground plant parts and on pest or alternative prey from the decomposer community below-ground (Settle et al. 1996; Scheu 2001). As a result, there are complex food web interactions in crops due to the presence of generalist predators, pests, other natural enemies and alternative prey not only on above-ground plant parts but also in the below-ground plant system. In Chapter 1, I show an example of a generalist predator that lives in the soil or litter layer and also preys on pests on the plant. This predator's life history inspired me to investigate the possibilities of connecting above-ground and litter food webs. Therefore, the question addressed in the rest of the dissertation was how connecting above-ground and below-ground plant systems can be used to improve biological control by generalist predators. Furthermore, I study how supplying natural enemies with alternative prey or food can improve biological control. In Chapters 2 and 3, I show how alternative prey added to the litter increases the biological control of thrips, a pest that causes damage on above-ground plant parts and spends part of its life cycle in the litter or soil. In Chapter 4, I show how a combination of generalist predator species and alternative foods in the litter and on above-ground plant parts affects the biological control of thrips.

In Chapter 1 I investigated how the generalist predator Balastium leanderi, which inhabits the below-ground plant system and forages on above-ground plant parts, was affected by feeding on a mixed diet of different species of important crop pests that affect above-ground plant parts. Several generalist predators have been shown to reproduce better on mixtures of prey species than on each prey species alone (Bilde & Toft 1994; Toft & Wise 1999; Messelink et al. 2008; Lefcheck et al. 2013; Marques et al. 2015). A special characteristic of B. leanderi is that it exhibits big-bang reproduction (semelparous reproduction), which means that females lay all their eggs at once and die soon after ovipositing. I show that B. leanderi females reproduce earlier and lay more eggs on a mixed diet of whitely eggs and spider mite eggs. Predator females that reproduce early consequently have higher population growth rates than late reproducers (Chapter 1). The effect of a mixed diet on B. leanderi is important for biological control because the mixed diet of whitely with spider mites boosts its population densities, possibly increasing the biological control of the three prey species (see Messelink et al. 2008 for an example). Further experiments should be carried out to confirm whether apparent competition mediated by B. leanderi occurs between the pests under cropping conditions.

In Chapter 2, I further investigated how connecting above-ground and below-ground food webs can serve to improve biocontrol with the predatory mite Amblyseius swirskii, which was known as an above-ground predator. I studied how the indirect interaction between the thrips and an alternative prey affected thrips control. Astigmatic mites (Cosmolaelaps n. sp. from the community of predators found in the litter in commercial rose crops. This predator benefits from an alternative prey added to the litter, the astigmatic mite C. lactis, and it also feeds on thrips. I studied the population dynamics of this predator and the pest, thrips. Similar to results in Chapter 2, I showed better thrips control by either the community of litter-inhabiting predator species or by
Cosmolaelaps n. sp. alone when astigmatic mites were added as alternative prey to the litter. I found that another species of predatory mite, Proctolaelaps pygmaeus, was abundant when the alternative prey was added to the community of predators, and that this predator feeds on the alternative prey C. lactis but not on thrips. It is not known to which extent the presence of predators like *P. pygmaeus* can affect thrips control by species such as *Cosmolaelaps* n. sp. Predator-predator interactions such as competition for food or intraguild predation among these litter-inhabiting predators should be addressed in future studies. Thus, in Chapter 3, I showed that litter-inhabiting predators can also mediate a negative interaction between prey, resulting in increased biological control of thrips.

Adding alternative food for predators does not always result in better pest control; some studies have shown that offering two prey to a generalist predator did result in increases in numbers of predators and decreases in the pest number, but not in decreases of plant damage or yield loss (Delisle et al. 2015; Jaworski et al. 2015). In Chapters 2 and 3, I showed that releasing *A. swirskii* or litter-inhabiting predators together with alternative prey in the litter results in decreases in thrips damage compared to treatments in which alternative prey was not added. However, damage to flowers, the marketable part of the crop, is more effective when the canopy-dwelling predator *A. swirskii* was released with alternative food (Chapter 2) than litter-inhabiting predators or *Cosmolaelaps* n. sp. with alternative prey (Chapter 3). Thus, the combination canopy-dwelling predators and litter-inhabiting predators with alternative prey might lead to lower damage of flowers.

Therefore, the question that I addressed in Chapter 4 was whether it would be possible to further decrease thrips damage when using different kinds of alternative food and a combination of canopy-dwelling and litter-inhabiting predators. I also tested thrips control when releasing predators preventively or only after thrips had established, which has shown to affect thrips control in other crops (Ramakers 1990; Kutuk & Yigit 2011; Kumar et al. 2015).

Previous experiments (Chapters 2 and 3) were done with litter added to the base of the plants, but the litter in commercial Dutch greenhouses is not found at the base of the plants, but further down, under the tables on which the plants are grown. In Chapter 4, I therefore tested the effects of addition of food under more realistic conditions, including the presence of a common soil predator that might improve or interfere with control. Thus, the distance between the litter and the plants was larger than in the previous chapters.

In the first experiment in Chapter 4, the question was whether adding alternative food either in the litter (Acarus siro) or on the plant (pollen) for a canopy-dwelling predator (*A. swirskii*) and litter-inhabiting predators would increase thrips control. In this experiment, thrips were released before the predators with alternative prey or pollen, and as a result, damage was too high to find differences among treatments. Decreases in thrips numbers were observed only during the last 3 weeks of the experiment, and only in the treatment with *A. swirskii* plus pollen (Chapter 4). All the leaves and flowers were damaged by thrips and I observed higher densities of thrips and higher plant damage during the first weeks on plants to which pollen was added as alternative food. This could have been an effect of apparent mutualism, of thrips feeding on the pollen, or of both. These results are similar to those obtained by Delisle et al. (2015), whom found more thrips damage on plants on which pollen was added than on plants without pollen. As explained above, apparent mutualism can occur when the shared predator becomes satiated or switches to feed on the alternative prey or food decreasing the attacks to the alternative prey (Murdoch 1969; Abrams & Matsuda 1996). This effect occurs in the short-term but can also occur in the long term when population densities fluctuate. Curiously, I did not observe apparent mutualism-like phenomena in Chapters 2 and 3. One obvious difference between the experiment described in Chapter 4 and those of the previous chapters was the distance between the litter and the above-ground plant parts. Possibly, *A. swirskii* did not commute as much between the litter and the canopy because of this increased distance, and instead concentrated their attacks initially on the alternative prey in the litter (apparent mutualism; Holt 1977; Abrams & Matsuda 1996). Holt (1984) studied models of prey that occurred in different habitats, but were attacked by the same predators. He predicted that negative effects of prey species on each other’s densities decrease when migration by the shared predator is slow, because it decouples the dynamics of the predators in the two habitats. This may have occurred in my experiments as well, which may explain the less effective control of thrips. Additionally, thrips perhaps did not only pupate in the litter under the tables with plants, but also on the tables at the base of the plant, where they would experience less predation by the litter predators. Summarizing, this experiment shows that the distance between the sites where alternative food or prey is supplied and the part of the crop where the pest occurs may be critical for efficient pest control. It also shows that by releasing thrips before predators, plant damage is too high in all treatments with or without predators and alternative food.

In a second experiment in Chapter 4, I tested the effect of the combination of predators (*A. swirskii* and litter-inhabiting predators) and two alternative foods (*A. siro* and pollen) on thrips control. The differences with the previous experiment were that a combination of predators and their own alternative food or prey were added simultaneously on the plant and in the litter, and that predators with alternative food were released before the thrips. Moreover, we included two frequencies of addition of pollen. The results of this experiment show that adding pollen to the plants for the plant-inhabiting predator *A. swirskii* leads to high predator densities and decreases...
General Discussion

in thrips densities and their damage (Chapter 4). Adding litter-inhabiting predators plus A. siro did not result in better thrips control than releases of only A. swirskii plus pollen. In fact, the presence of litter-inhabiting predators plus alternative prey did not have any effect on thrips densities. This is in contrast with the findings of Chapter 3 when releasing another litter-inhabiting predator, Cosmoseiulus alops n. sp. plus alternative prey in the litter resulted in a decrease in thrips numbers and thrips plant damage (Chapter 3). A possible reason for litter-inhabiting predators not contributing substantially to the control thrips is that the litter-inhabiting predators were far removed from the above-ground plant parts in Chapter 4. However, the presence of litter-inhabiting predators plus the alternative prey did not have negative effects on thrips control by A. swirskii (Chapter 4). Future experiments should investigate whether placing some litter with litter-inhabiting predators plus alternative prey at the base of the rose plants would result in better control. Many pest species spend part of their life cycles in the litter or soil (Wahab 2010), others drop to the soil when threatened (Losey & Denno 1998). Thus, having high quantities of litter or soil-inhabiting predators could contribute to the control of various pest species in many crops.

Another aspect that should receive more attention in future studies is the timing at which alternative food should be added to the crop. Many studies have shown that adding alternative food can improve biological control (McMurtry & Scriven 1966; Knight et al. 1979; van Rijn et al. 2002; Maoz et al. 2009; Nomikou et al. 2010; Adar et al. 2014; Duarte et al. 2015), but none have investigated the amounts and supply frequencies on biological control. In the second experiment presented in Chapter 4, two frequencies of pollen addition were tested. In one treatment, pollen was added during 4 weeks out of the 14 weeks of the experiment. In other treatments pollen for A. swirskii was added weekly. There were no differences among treatments concerning thrips numbers or thrips plant damage. However, there were differences in the number of A. swirskii. Leaves of plants to which pollen was added every week harboured significantly more predators than plants with the interrupted feeding treatment. Future studies should further explore ‘optimal’ frequencies of addition of alternative food on crop protection.

Summarizing, in this thesis, I present experiments that contribute to the test of food web theory. I studied aspects such as apparent competition, apparent mutualism, effects of combined releases of predators on prey on above-ground and below-ground plant systems. The results in this thesis contribute to the understanding of the dynamics of above-ground and below-ground food webs and they are first steps in the development of a new biological control strategy in ornamentals, which is the addition of alternative food for predators in the litter. Comparing experimental results and existing theory of population dynamics can be challenging because of differences in time scale. Experiments are usually short, but most theory is based on long-term equilibrium dynamics (Briggs & Borer 2005). The experiments performed in this thesis were long enough to record positive and negative effects of the addition of alternative food or prey on pest populations and on plant damage, hence they succeed in assessing whether such effects as apparent competition and apparent mutualism do occur. Moreover, they reveal connections between above-ground and below-ground food webs through the foraging of generalist predators. A question that arises at this point is how these findings can help growers to enhance biological control. In Chapter 2, I proposed a novel way to enhance pest control in ornamental crops by supplying alternative food in the litter. Adding alternative prey in the litter at the base of the plant, far from the commercial plant parts may be an important contribution to sustainable production of ornamentals. It has the advantage that no residues of alternative food will be left on the marketable plant parts (Chapter 2 and 3). As a conclusion, links can be established between above-ground plant pests and the litter food web and such links can benefit above-ground and below-ground predators, resulting in an increase of predator densities and enhanced biological control. Thus, connections between above-ground and below-ground food webs associated with plants may play a key role not only in natural communities (van der Putten et al. 2001; A’Bear et al. 2014), but also in agricultural systems. They should therefore receive more attention when designing biological control programs.

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1 – Generalist red velvet mite predator (*Balaustium leanderi*) performs better on a mixed diet
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  KMC, LSF, RFC & CDR – planned the experiments; KMC & LSF – conducted experiments; KMC, AJ & MWS – analysed the data; KMC, AJ & MWS – wrote the manuscript

2 – Supplying high-quality alternative prey in the litter increases control of an above-ground plant pest by a generalist predator
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3 – Alternative food for litter-inhabiting predators decreases pest densities and above-ground plant damage
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  KMC & AJ – planned the experiments; KMC DRR, FE & FF – conducted experiments; KMC & AJ – analysed the data; KMC & AJ – wrote the manuscript

4 – Single and combined predator releases with alternative food increases thrips control in an ornamental crop
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  KMC, AL, GJM & AJ – planned the experiments; KMC, AL & MVAD – conducted experiments; KMC, GJM & AJ – analysed the data; KMC, AL, GJM & AJ – wrote the manuscript

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Summary

Arthropod food webs associated with plants are commonly composed of several species of herbivores, the detritivore community, and specialist and generalist predators and parasitoids that feed on the first two groups and on each other. In the past, specialist natural enemies were preferably used for biological control because they are adapted to their prey. However, they cannot persist in a crop when these prey are scarce or absent. Thus, specialist natural enemies can only be successfully released after the pest has invaded the crop, or must be released repeatedly, which can be costly. In contrast, populations of generalist predators can persist in crops by feeding on different species of pests, plant-provided food sources such as honey and pollen, and on organisms of the detritivore community. Thus, generalist natural enemies mediate a number of direct and indirect interactions within and between arthropod communities associated with plants. These interactions can have positive or negative effects on biological control. For example, a prey species is often attacked by several species of natural enemies, which thus compete for this prey. As a consequence, different species of natural enemies can have additive effects on prey populations; their combined effects on a prey population equal the sum of the effects of the different natural enemy species, thereby increasing biological control. Multiple natural enemies can also disrupt biological control, for example because of interactions such as intraguild predation among natural enemies, or because the response of the prey to one species of natural enemy decreases the risk of being attacked by the other species. Furthermore, the combined effect of several species of natural enemies can cause further decreases in prey densities than their additive effect, for example because they feed on different stages of the prey, or because the response of prey to one natural enemy species makes them more vulnerable to the other species. Thus, in biological control the effect of multiple natural enemies versus the effect of a single natural enemy varies and must be investigated before implementing biological control strategies in crops. Studies that show interactions among predators that have a positive effect on pest control involve systems in which predators attack the pest at different locations or on different plant parts, in different seasons, or at different life stages. An aspect that has not received enough attention and I investigated in this thesis is the effect of the addition of alternative food for different generalist predators (on the canopy and in the litter) on biological control.

The effects of adding alternative food such as pollen to increase biological control by a generalist predator has been widely investigated. There can be positive effects
of adding alternative food or prey on biological control because it results in an increase in predator densities in the long term, after one or a few predator generations. Alternative prey and food thus affect the density of a pest indirectly by changing the population densities of the generalist natural enemies. Adding alternative food or prey to a system of one predator and one pest species increases the density of the predator, and this numerical response leads to a decrease in pest densities. This is called ‘apparent competition’, because the effect of the alternative food on to the pest is reminiscent of competition for resources. These negative effects of alternative food or prey on the pest are a consequence of the numerical response of the predators, hence, become manifest after a few generations of the predator. In the short term, when the populations of predators have not reached dynamic equilibria, the natural enemy-mediated indirect interaction may cause the opposite effect: the addition of alternative prey or food can result in satiation of the predator population, which will decrease predation of the pest population. In this case, the pest benefits from the addition of alternative food (so-called ‘apparent mutualism’). Such effects may also occur repeatedly in predator-prey systems that show long-term persistent fluctuations. Hence, positive or negative consequences can be expected when using generalist predators for biological control. Studying the effects of the interactions of generalist predators with other members of the food webs is therefore important to predict the effects of releasing these predators and supplying them with alternative food on pest control.

Generalist predators can also connect above-ground and below-ground food webs when they feed on prey and other food sources in both food webs. The effect of the connection of these food webs by generalist predators on pest control has not received much attention, especially in the case of ornamentals. Thus, the main research question of this thesis was how interactions between above-ground and litter food webs affect biological control. In Chapter 1, I investigated the effect of a mixed diet of above-ground plant pests (spider mites, thrips, whiteflies) on the life history traits of a generalist below-ground predator, *Balaustium leanderi*, which also forages on the canopy of crop plants. I recorded its life history traits (reproductive performance, survival and development) when fed on mixed diets of three pest species that inhabit above-ground plant parts. The predators reproduce more and faster when feeding on this mixed diet than on single diets. In Chapter 2, I investigated *Amblyseius swirskii*, another generalist predator that mainly inhabits the canopy. I show that it makes excursions to the litter layer to feed or disperse. From this chapter on, I focused on the control of the western flower thrips *Frankliniella occidentalis* because it is one of the most damaging pests in ornamentals. Like many other pests, this thrips spends its life cycle partly on above-ground plant parts and partly in the litter or soil. In the specific case of thrips, they move to the soil or litter to pupate. I show that by adding astigmatic mites (Acari: Astigmata) as alternative prey for the predators to the litter, the densities of *A. swirskii* were boosted, resulting in decreases of thrips densities (apparent competition) and decreases in plant damage. In Chapter 3, I investigated the effect of predators that inhabit the litter of commercial rose production greenhouses on thrips control. I show that supplying astigmatic mites in the litter as alternative food for either a community of predators or for a single predator species, *Cosmoalephas n. sp.*, results in decreases in thrips densities (apparent competition) and plant damage.

In commercial crops, canopy-dwelling predators (such as *A. swirskii*) are commonly released to control pests, and these may interact with the litter-inhabiting predators that are already present (such as *Macrocheles robustus*, *Stratiolaelaps scimitus* and *Cosmolaelaps n. sp.*), and this might affect biological control. For this reason, I investigated control of thrips by the combination of the canopy-dwelling predator *A. swirskii* and litter-inhabiting predatory mite species such as *M. robustus* and *S. scimitus* in Chapter 4. Because pests such as thrips inhabit above-ground plant parts and the litter or soil, attempts have been made to increase thrips control by combining canopy-dwelling predators and soil-inhabiting predators. The novelty here is that I investigated whether biological control can be enhanced by supplying different alternative foods for canopy-dwelling and litter-inhabiting predators (pollen and astigmatic mites, respectively). Moreover I assessed whether the presence of thrips before predator releases would affect biological control and whether the frequency of addition of pollen for canopy-dwelling predators would affect thrips control. I conclude that adding pollen on the plants for canopy-dwelling predators resulted in the best thrips control (as in apparent competition), either with or without litter-dwelling predators, and these latter predators did not interfere with thrips control. In this experiment, litter was placed on the soil, under the tables on which the plants are grown, as is customary in commercial rose production. Because the litter predators were released in that litter, the distance from the canopy was rather large. Further studies could therefore include treatments in which litter-dwelling predators with alternative food are added at the base of the plant, just below the canopy. This would create a tighter link between the food webs above-ground and in the litter. The study of these litter-dwelling predators to control other pests deserves further study because they are well adapted to the crop and their populations can be boosted with alternative food (Chapter 3). The release of predators after thrips was introduced proved detrimental for biological control, with high plant damage in all the treatments. In contrast, plants were protected from thrips damage when the predators were released with pollen before introducing the thrips. Furthermore, I found no differences in thrips densities or thrips damage either by adding pollen weekly or when...
interrupting the addition of pollen. However, there were significantly more predators on plants that received pollen weekly. Concluding, in CHAPTER 4 I show that in order to decrease flower damage by thrips in ornamentals such as roses, canopy-dwelling predators should be released before thrips invasions. Also, pollen should be added on a weekly basis. I suggest that more studies on the frequency and quantity of supplemented alternative food should be conducted. Moreover, the role of litter-dwelling predators in control of thrips and other pests in crops with higher damage thresholds as in ornamentals deserves further study.

Thus, in this thesis I present different aspects that can be used by biological control practitioners. I also demonstrate that greenhouse experiments can help to test ecological theories: I show that adding alternative food for generalist predators mainly results in apparent competition, apparent mutualism was found in few occasions for short periods of time, when the pest was present before the predators were released. Based on this thesis I recommend two factors that deserve further theoretical exploration. The first is that the effect of mixed diets on apparent competition should be further investigated. The second is the effect of the frequency of supplying alternative food on the dynamics of the pest and natural enemy. I also show that the interactions between above-ground and below-ground food webs affect pest densities. In this regard, I recommend that theoretical approaches could be directed to study the impact of multiple generalist predators and food sources in above-ground and below-ground plant parts on pest control.

Summarizing, I conclude that litter and canopy food webs can be linked and this can result in increased biological control in an ornamental crop and that greenhouse experiments evaluating the population dynamics of multiple predators with multiple food sources are crucial for the development of new biological control strategies. At the same time, such experiments are excellent test cases for ecological theories.

**Samenvatting**

Voedselwebben van geleedpotigen op planten bestaan meestal uit diverse soorten plantenetters, detritivoren en specialistische en generalistische natuurlijke vijanden: predators en parasieten, die zich voeden met leden van de eerste twee groepen en met elkaar. Gewasbeschermers hadden in het verleden een voorkeur voor het gebruik van specialistische natuurlijke vijanden voor biologische plaagbestrijding omdat die zijn aangepast aan hun prooi, maar zulke specialisten kunnen zich niet handhaven in het gewas als de plaagdichtheid laag is. Ze kunnen daarom alleen maar worden losgelaten nadat de plaat het gewas al heeft geïnvaderd, of ze moeten herhaaldelijk worden losgelaten, hetgeen kosten met zich meebrengt. Generalistische predators daarentegen kunnen zich in het gewas handhaven door dat zij zich kunnen voeden met diverse plaagsoorten, met plantaardig voedsel zoals honing en stuifmeel, en met detritivoren. Generalisten veroorzaken hierdoor allerlei directe en indirecte interacties binnen het voedselweb geassocieerd met planten.

Deze interacties kunnen zowel positieve als negatieve gevolgen hebben voor biologische plaagbestrijding. Bijvoorbeeld, een prooisoort kan vaak door meerdere soorten predators worden aangevallen, waardoor die predators concurreren om deze prooi. Ten gevolge hiervan kunnen verschillende soorten natuurlijke vijanden een additief effect hebben op prooidichtheden: prooidichtheden in aanwezigheid van verschillende soorten predators zijn dan lager dan in de aanwezigheid van iedere vijand apart. Er kunnen echter ook andere interacties dan concurrentie om voedsel optreden tussen de verschillende predators. Zo kunnen ze bijvoorbeeld elkaar aanvallen. Ook kan het effect van een soort predator op het gedrag van de prooi (bijvoorbeeld vluchtgedrag) ertoe leiden dat andere soorten vijanden meer moeite hebben met het vangen van de prooi. Door deze indirecte interacties kan biologische bestrijding met meerdere soorten natuurlijke vijanden minder efficient zijn dan met iedere vijand apart. Als laatste kan het zo zijn, dat het gebruik van verschillende soorten vijanden een synergistisch effect heeft op plaagbestrijding, doordat de vijanden bijvoorbeeld verschillende ontwikkelingsstadia van de prooi aanvallen, of doordat ze gedragsveranderingen in de prooi veroorzaken waardoor het makkelijker voor andere vijanden wordt om de prooi te vangen.

Voor efficiënte biologische bestrijding is het nodig dat de effecten van verschillende natuurlijke vijanden, alleen of gezamenlijk, op plaagdichtheden worden onderzocht. Betere plaagbestrijding door het gebruik van meerdere natuurlijke vijanden wordt vooral gevonden in systemen waarin de diverse vijanden de plaat op verschil-
lende plekken aanvallen, bijvoorbeeld op verschillende delen van de plant, waar ze verschillende stadia van de plagaanvallen, of in systemen waarin de vijanden in verschillende seizoenen actief zijn. Een aspect dat tot nu toe nog niet veel onderzocht is, en wat ik in dit proefschrift onderzoek, is het effect op biologische bestrijding van het toevoegen van alternatief voedsel voor verschillende soorten natuurlijke vijanden. Dit voedsel wordt zowel toegediend op de bovengrondse plantendelen als op de strooisellaag aan de voet van de planten.

De effecten van het toedienen van alternatief voedsel, zoals pollen, op biologische bestrijding zijn al vaak onderzocht. Er kan een positief effect van het toevoegen van pollen op biologische bestrijding zijn omdat het na een aantal generaties van natuurlijke vijanden leidt tot hogere dichtheden van de vijanden. Het alternatieve voedsel zorgt dan indirect voor lagere plaagdichtheden door hogere dichtheden van de vijanden via de numerieke respons. Dit fenomeen wordt wel ‘apparent competition’ genoemd, omdat de dynamiek van de plaag lijkt op die van een populatie die concurrereert met een andere populatie, in dit geval lijkt het of de plaag concurreret met het alternatieve voedsel. Op kortere termijn echter kan de indirecte interactie tussen het alternatieve voedsel en de plaag leiden tot het tegenovergestelde effect: het toevoegen van alternatief voedsel leidt tot verzadiging van de aanwezige predators en dat leidt vervolgens tot lagere predatie van de plaagpopulatie. In dat geval profiteert de plaag dus van het toevoegen van het alternatieve voedsel (zogenaamd ‘apparent mutualism’). Zulke verzadigingseffecten kunnen ook herhaaldelijk optreden wanneer de dynamica van de natuurlijke vijand en de plaag persisterende oscilaties vertoont. Concluderend, het toevoegen van alternatief voedsel voor predators kan tot positieve, maar ook tot negatieve effecten op biologische bestrijding leiden. Het is daarom belangrijk voor efficiënte en milieuvriendelijke biologische bestrijding om de effecten van de interacties van generalistische predators met andere soorten in het voedselweb te effecten.

Generalistische predators kunnen ook een indirecte interactie tussen verschillende voedselwebben bewerkstelligen, bijvoorbeeld het voedselweb op de bovengrondse plantendelen en dat onder de grond. Hoe biologische bestrijding wordt beïnvloed doordat generalistische predators deze voedselwebben verbinden is nog niet uitgebreid bestudeerd, al helemaal niet in siergewassen. De voornaamste onderzoeksvraag van dit proefschrift is daarom hoe biologische bestrijding van een plantenplaag wordt beïnvloed door de interacties tussen het voedselweb op de bovengrondse plantendelen en het voedselweb in de strooisellaag aan de voet van de plant. In HOOFDSTUK 1 onderzoek ik het effect van een gemengd diet van verschillende bovengrondse plantenplagen (spinfrijtmijten, wittevlieg en trips) op de levensgeschiedenis van de generalistische predator Balaustium leanderi. Deze roofmijt leeft voornamelijk ondergronds, maar zoekt ook naar voedsel op bovengrondse planten-

delen. De levensgeschiedenissenmerken die ik bestudeerde waren reproduktie, overleving en ontwikkeling. De predators blijken meer en sneller te reproduceren als ze zich voeden met een gemengd diet dan op een dieet van ieder van de drie soorten afzonderlijk. In HOOFDSTUK 2 bestudeer ik Amblyseius swirskii, een andere generalistische roofmijt, die voornamelijk op bovengrondse plantendelen leeft. Ik laat zien dat deze predator excursies naar de strooisellaag maakt om daar te voeden of te dispergeren. Vanaf dit hoofdstuk concentreer ik me op de Californische trips Frankliniella occidentalis omdat het een van de belangrijkste plagen in de sierteelt is. Zoals vele andere plagen, brengt deze trips een deel van zijn levenscyclus in de strooisellaag door, in dit geval om te verpoppen. De trips valt echter vooral bovengrondse plantendelen aan. Ik laat zien dat het toevoegen van alternatief voedsel in de vorm van astigmate mijten (Acarina: Astigmata) aan de strooisellaag resulteerde in hogere dichtheden van A. swirskii, met als gevolg dat trips-dichtheden en de schade aan planten afnamen (‘apparent competition’). In HOOFDSTUK 3 onderzoek ik het effect van predators uit de strooisellaag van commerciële rozenkassen op de bestrijding van trips. Ik toon aan dat de roofmijt Cosmolelaeaps n. sp., alleen of in combinatie met andere bodembewonende roofmijten, tripspopulaties beter bestrijdt en plantenschade meer beperkt als astigmate mijten aan de strooisellaag werden toegevoegd als voedsel voor deze predators (‘apparent competition’).

Bladbewonende rovers zoals A. swirski worden vaak losgelaten in commerciële gewassen om plag te bestrijden, maar deze rovers kunnen interacties aangaan met de predators die zich in de strooisellaag bevinden, zoals Macrocheles robustulus, Stratiolaeaps scimitus en Cosmolelaeaps n. sp., en dit kan biologische bestrijding beïnvloeden. Omdat plaaginsecten zoals thrips zowel bovengrondse plantendelen als de strooisellaag bewonen, zijn er verschillende pogingen geweest om trips te controleren met een combinatie van plantbewonende en bodembewonende natuurlijke vijanden. In HOOFDSTUK 4 onderzoek ik de bestrijding van trips met een combinatie van A. swirski en bodembewonende roofmijten zoals M. robustulus en S. scimitus. Nieuw in deze experimenten is dat ik verschillende typen alternatief voedsel toedienen voor de twee typen rovers (pollen bovengrond en astigmaten mijten in de strooisellaag). Ik onderzoek bovendien het effect van het loslaten van de rovers voor dan wel na een tripsinfecie, en van de frequentie waarmee pollen wordt toegevoegd op tripsbestrijding. De voornaamste conclusie is dat het toevoegen van pollen voor de bladbewonende rovers resulteert in de beste tripsbestrijding (zoals bij ‘apparent competition’), onafhankelijk van de aanwezigheid van rovers in de strooisellaag. Bovendien blijken de rovers in de strooisellaag de bestrijding van trips niet te beïnvloeden. Zoals gebruikelijk in commerciële kweken, bevond de strooisellaag in dit experiment zich op de bodem, onder de tafels waarop de planten groeien. Hierdoor was de afstand van de planten tot die strooisellaag vrij groot. Toekomstige experi-
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menten zouden daarom kunnen achterhalen wat het effect is van het toevoegen van strooisel met rovers aan de voet van de planten, hetgeen zou kunnen resulteren in een sterkere verbinding tussen de beide voedselwebben. Bestrijding van andere planten door de predators die in de strooisellaag leven verdient meer studie omdat ze goed zijn aangepast aan het gewas en omdat hun dichtheden kunnen worden verhoogd door toediening van alternatief voedsel (Hoofdstuk 3). Het loslaten van de predators nadat tripsaantasting was opgetreden resulterde in onvoldoende bestrijding, met veel schade aan de planten. Wanneer predators werden losgelaten en bijgevoerd met pollen voor de tripsaantasting, waren planten wel beschermd. Ik vond geen verschil in tripsdichtheden en schade wanneer pollen wekelijks werd toegevoegd en wanneer die toediening werd onderbroken. Er waren echter wel significant meer rovers aanwezig op de planten die continu pollen ontvingen. Concluderend, laat ik in Hoofdstuk 4 zien dat bladbewonende rovers moeten worden losgelaten voordat trips invaert om schade aan bloemen van siergewassen zoals roos te verminderen. Bovendien zou pollen wekelijks moeten worden toegevoegd. Ik geef aan dat meer studie nodig is naar de frequentie van toediening en de kwaliteit van alternatief voedsel. Ook de rol van predators in de strooisellaag bij de bestrijding van trips en andere plagen verdient meer onderzoek.

In dit proefschrift presenteer ik diverse methoden die kunnen worden gebruikt bij biologische bestrijding. Ik laat ook zien dat kasexperimenten kunnen helpen bij het testen van ecologische theorie: ik toon aan dat het toevoegen van alternatief voedsel voor generalistische predators voornamelijk resultert in ‘apparent competition’; ‘apparent mutualism’ werd af en toe waargenomen, en gedurende korte perioden, vooral wanneer de plaat aanwezig was voor het doel van de predators werden losgelaten. Op basis van dit proefschrift, suggereer ik twee factoren die meer theoretische aandacht verdienen: (1) het effect van gemengde diëten op ‘apparent competition’ en (2) het effect van de frequentie van toediening van alternatief voedsel op de dynamica van predators en prooien. Ik laat ook zien dat de interacties tussen bovengrondse en ondergrondse voedselwebben effect hebben op plaagdichtheden. Met betrekking tot dit laatste onderwerp, beveel ik theoretische studies aan over het effect van meerdere soorten van generalistische predators en het toedienen van voedsel bovengronds en ondergronds op plaagbestrijding.

Samenvattend, concluder ik dat voedselwebben in de strooisellaag en op de bovengrondse plantendelen kunnen worden verbonden en dat dit kan resulteren in betere biologische bestrijding in een siergewas. Kasexperimenten naar de dynamica van meerdere predatorsoorten met meerdere voedselbronnen zijn cruciaal voor het ontwikkelen van nieuwe bestrijdingsmethoden. Zulke experimenten vormen tegelijkertijd goede tests voor ecologische theorie.

Curriculum vitae

Karen was born in Bogotá, Colombia, surrounded by her beloved – and luckily a bit crazy and adventurous – relatives. She went to a catholic primary school in Soacha, a town near Bogotá. Karen and her family moved to Bogotá when she was 12 years old. There she attended a public technical high school. After graduating from high school, Karen travelled to the USA to work and to learn English. In 2002, Karen returned to Colombia to study a bachelor’s degree in Applied Biology at the Nueva Granada Military University (UMNG). Karen was, is and will be a pacifist, so going to a military university may sound paradoxical. However, the UMNG was affordable, and their biology program focused on the important role biodiversity plays in agriculture.

Karen became especially interested in the subject of biological control: visiting and conducting experiments in ornamental and aromatic crops, Karen observed that in crops with low pesticide use, there was a higher diversity of beneficial insects and mites, and a better quality of life for the growers. This had a strong impact on Karen’s life. That’s why she decided to pursue a master’s degree focused on plant protection and biological control at the UMNG. During her postgraduate studies, Karen continued working as a research assistant for the Colombian Association of Flower Growers (ASOCOLFLORES). Her master’s thesis dealt with the integrated pest management in rose crops.

In 2008, Karen attended a workshop on agricultural acarology at Ohio State University. There she met Prof. Maurice Sabelis. He accepted Karen in his team. In 2010, she earned a scholarship from the Colombian Department of Science and Technology (COLCIENCIAS) to pursue a PhD program. In the year 2011, Karen did an internship in a biological control company, met her soul mate, and began her PhD research.

Karen now works as an Environmental Risk Evaluator at the Dutch Board for the Authorization of Plant Protection Products and Biocides (CTGB). In her new role, Karen wishes to continue highlighting the importance of biodiversity for sustainable agriculture.

Publications


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