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*Linking litter and canopy food webs to protect ornamental crops*

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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).

Prey	Overall effect and comparisons	df	Likelihood ratio	P
Whitefly eggs	Overall	2	109.0	<0.0001
	Larvae-Nymphs	1	77.7	<0.0001
	Nymphs-Adults	1	24.4	<0.0001
	Adults-Larvae	1	105.5	<0.0001
Thrips larvae	Overall	2	30.2	<0.0001
	Larvae-Nymphs	1	1.11	0.29
	Nymphs-Adults	1	20.7	<0.0001
	Adults-Larvae	1	27.3	<0.0001
Spider mite eggs	Overall	2	187.8	<0.0001
	Larvae-Nymphs	1	135.7	<0.0001
	Nymphs-Adults	1	56.9	<0.0001
	Adults-Larvae	1	181.5	<0.0001

**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).

Predator stage	Prey	Comparison	df	Likelihood ratio	P
Larva	Whitefly eggs	W vs. W+T	1	46.9	<0.0001
	Spider mite eggs	S vs. S+T	1	77.2	<0.0001
Nymph	Whitefly eggs	W vs. W+T	1	15.9	<0.0001
	Spider mite eggs	S vs. S+T	1	44.9	<0.0001
Adult	Whitefly eggs	W vs. W+T	1	14.4	<0.0001
	Spider mite eggs	S vs. S+T	1	63.1	<0.0001



## Supplying high-quality alternative prey in the litter increases control of an above-ground plant pest by a generalist predator

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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 (astigmatic 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 astigmatic 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.

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## 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 & Murai 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-

rior or inferior quality compared to the pest. Finally, we carried out experiments in the laboratory and greenhouse to test whether control of thrips was improved by supplying alternative prey for their predators in the litter.

## Materials and methods

### Rearing methods

Rose plants for cut flower production were purchased when they were 4 weeks old and kept in acclimatized rooms or in a greenhouse. To avoid contamination with arthropods, we removed all leaves and washed the stems with running tap water. Subsequently, the stems and roots were allowed to regrow in an acclimatized room (25 °C, RH 60%, photoperiod L16:D8, Sylvania GRO-LUX F58W/GRO 5FT T8 58W) or a greenhouse compartment (22 °C, RH 70%, photoperiod L14:D10) inside cages. They were either planted in pots (26 cm diameter, 25 cm high) with peat as substrate (50% coco peat, 15% white peat, 35% frozen black peat; Jongkind grond BV, Aalsmeer, The Netherlands) or in a rockwool strip (Grodan® Delta) inside a plastic tray (42 × 8 × 30). Plants were watered twice a week, and macro- and micronutrients (0.5 g N-P-K and 0.5 g micronutrients mix/200-500 ml of water/plant) were applied once a week.

*Frankliniella occidentalis* came from the stock colony of Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands), where they had been reared on runner bean pods *Phaseolus vulgaris* L. plus *Typha latifolia* L. pollen for more than 10 years. The thrips were reared in the laboratory (at 23 °C, RH 65%) in plastic containers (8 × 8 × 12 cm), with openings covered with mite-proof mesh (30 µm) for ventilation (adapted from Loomans & Murai 1997). Around 200 adult thrips were introduced into each container with four half bean pods, in which the females laid eggs for 1 day. Subsequently, the pods were replaced with fresh ones for further egg laying. The egg-infested pods were transferred to clean rearing containers, giving rise to individuals of the same age. Young first-instar thrips were taken from these containers and used for experiments on food quality.

*Amblyseius swirskii* used for laboratory experiments were originally collected from cotton in Redavim, Israel (Nomikou et al. 2001). They were reared on plastic arenas (8 × 15 cm), placed on a wet sponge in a plastic tray containing water, following Overmeer (1985). Strips of wet tissue were placed along the periphery of the arena to provide water. A tent-shaped piece of plastic sheet (1–2 cm<sup>2</sup>) was placed on each arena and functioned as a shelter for the mites. A few cotton wool threads were put underneath the shelter as oviposition substrates. Cultures were fed with *T. latifolia* pollen. To produce cohorts of predators of the same age, the threads were transferred to a new rearing arena every 1-2 days. Ten days later, adults from these arenas were used for experiments. For greenhouse experiments, commercially available predators were used (Swirski-mite®, Koppert Biological Systems).

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 rearings of *A. swirskii* (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 swirskii*

Because *A. swirskii* 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. swirskii* 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 10 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 nlme 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

(Yong 2004). There were two treatments, cages to which *A. swirskii* was added and cages with *A. swirskii* plus alternative prey (*T. putrescentiae*) added. 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 / 1 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  $\mu\text{m}$ ) 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

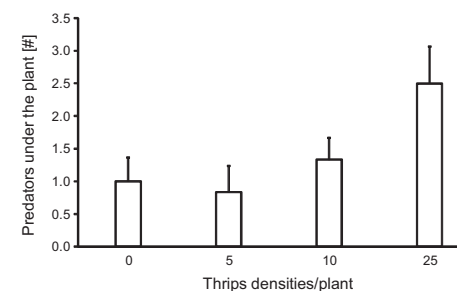
A pilot experiment showed that high densities of *A. swirskii* controlled thrips and decreased the thrips damage of flowers after 4 weeks (SUPPLEMENTARY MATERIAL 2.1). We therefore investigated whether adding alternative food to the litter could boost densities of this predator to sufficiently high levels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

### Movement of *Amblyseius swirskii*

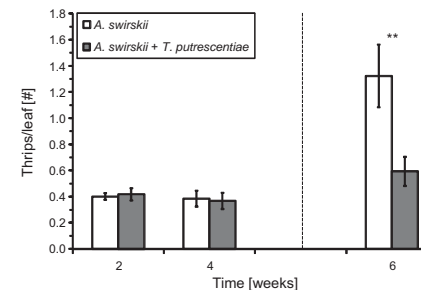
Of the five mites released per plant on the above-ground parts, we recaptured on average 37.5% (s.e. 7.2%). The majority of these (1.4 mites/replicate) were found in the Petri dishes with alternative prey; the others were found on the plants. Most probably, the rest of the mites were inside the rock 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. = 3,  $\chi^2 = 6.34$ ,  $P = 0.10$ ). 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 ( $\pm$  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,  $P > 0.05$ ).

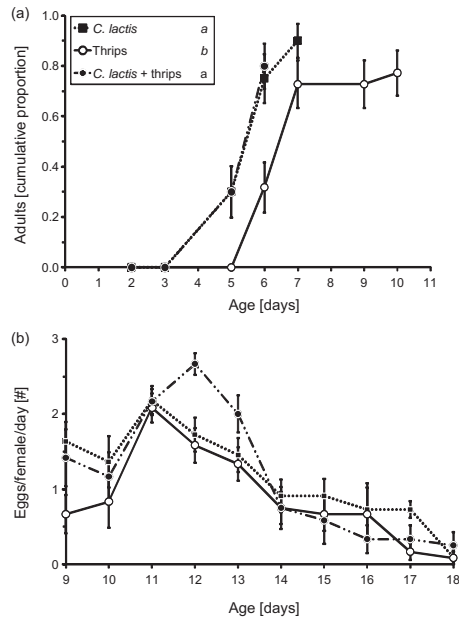


**FIGURE 2.2.** Effect of addition of astigmatic mites (*Tyrophagus putrescentiae*) as alternative prey in the litter layer under laboratory conditions on average numbers of thrips/leaf ( $\pm$  s.e.). Data of the second and fourth weeks correspond to samplings of 10 leaves per cage; data of the sixth 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:  $\chi^2 = 3.03$ ,  $P = 0.22$ ).

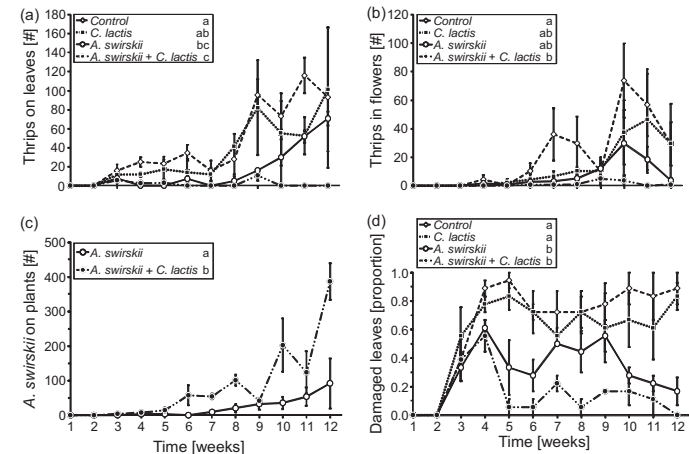


**FIGURE 2.3.** (a) Development of juveniles of the predatory mite *Amblyseius swirskii* on a diet of either *Carpoglyphus lactis*, thrips, or *C. lactis* plus thrips. Shown are the cumulative proportions of juveniles that developed into adults ( $\pm$  s.e.). Different letters in the legend represent significant differences in developmental rate among treatments (contrasts with general linear hypothesis testing after Cox proportional hazards,  $P < 0.05$ ). Differences in survival until adulthood were not significant ( $P > 0.05$ ). (b) Average numbers of eggs per female per day ( $\pm$  s.e.) of young female *A. swirskii* during 10 days on a diet of *C. lactis*, thrips or *C. lactis* plus thrips. There was no significant effect of diet on oviposition (LME,  $P > 0.05$ ).

### 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-



**FIGURE 2.4.** Effect of addition of *Carpoglyphus lactis* to a sterilized litter layer as alternative prey for *Amblyseius swirskii* on thrips control under greenhouse conditions. (a) The average number of thrips per leaf on rose plants ( $\pm$  s.e.) inside cages. Thrips were released in the second week and larvae and adult thrips were sampled from the third week on. (b) The average number of thrips in flowers inside the same cages as (a). (c) The average number of *A. swirskii* ( $\pm$  s.e., all stages). (d) The average proportion of damaged leaves ( $\pm$  s.e.) per week. Different letters in the legend represent significant differences among treatments through time (contrasts with general linear hypothesis testing after LME,  $P < 0.05$ ).



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; Pijnakker 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 (Messelink & 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/m<sup>2</sup> of greenhouse, commercial recommendation is 100 mites/m<sup>2</sup>), 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 densities 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% white 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*/m<sup>2</sup>, 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 ( $\sqrt{x}$  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  $\pm$  s.e. =  $0.058 \pm 0.039$  vs.  $0.24 \pm 0.10$  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:  $2.23 \pm 0.59$  thrips/flower, without predators:  $13.2 \pm 5.0$ ; 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  $157.2 (\pm 55.4)$  *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



FIGURE S2.1.1. Effect of high densities of predators on thrips damage to rose petals. Left: petals from flowers of plants treated with high densities of *Amblyseius swirskii*. Right: control treatment with only thrips.

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 flonicamid (Teppeki, 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

**Table S2.1.1.** Schedule of releases and samplings of the western flower thrips *Frankliniella occidentalis*, predatory mites (*Amblyseius swirskii*) and alternative prey (*Tyrophagus putrescentiae*) when assessing the effect of adding alternative prey.

Time (weeks)	1	2	3	4	5	6	7	8	9	10
<i>A. swirskii</i> <sup>a</sup>	105	105	30							
<i>F. occidentalis</i> <sup>b</sup>		5+10	5+15	10+15		20+20		10+0		
<i>T. putrescentiae</i> <sup>c</sup>		100	300	3000	3000	3000				
Sampling						√	√	√	√	√

<sup>a</sup> Number of adult females released per week.

<sup>b</sup> Numbers of a mixture of adults + juveniles released.

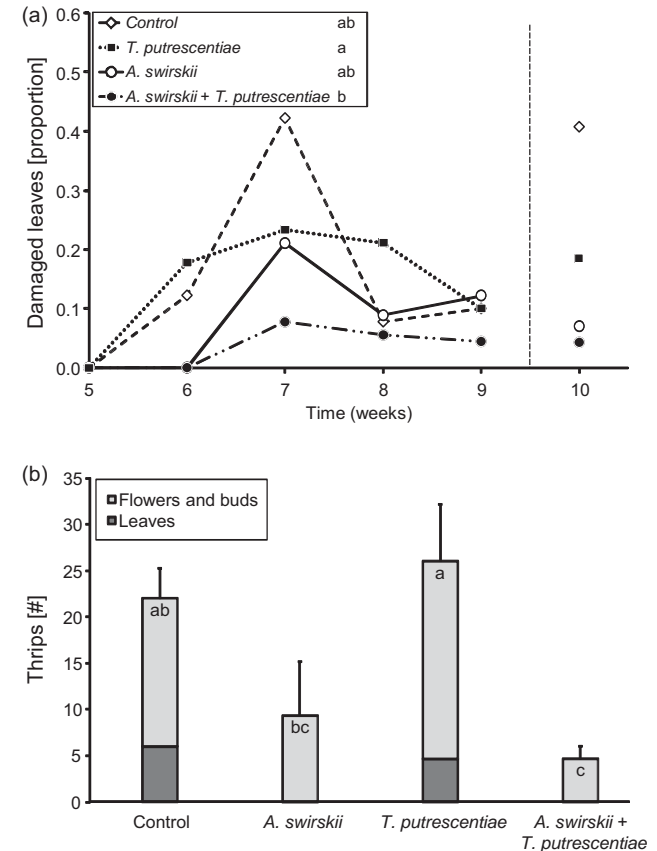
<sup>c</sup> Number of mites released with bran, adult females were counted, immatures were also present but not counted.

factor. The numbers of thrips on leaves were  $\sqrt{x}$  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,  $\chi^2 = 11.2$ ,  $P = 0.01$ ), 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:  $F_{3,8} = 1.75$ ,  $P = 0.23$ ). Densities of thrips on leaves were low (FIGURE S2.1.2b) and did not differ significantly among treatments (GLM:  $F_{3,8} = 2.61$ ,  $P = 0.12$ ). In contrast, the numbers of thrips in flowers and buds were high (FIGURE S2.1.2b) and differed among treatments (GLM: d.f. = 3,  $\chi^2 = 40.1$ ,  $P < 0.0001$ ), 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 ( $71.3 \pm 27.4$  vs.  $16.7 \pm 4.8$ ; GLM:  $F_{1,4} = 6.15$ ,  $P = 0.068$ ).

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.



**Figure S2.1.2.** Effect of addition of the astigmatic mite *Tyrophagus putrescentiae* as alternative prey in the litter layer under greenhouse conditions on the proportion of damaged leaves **(a)**. Each point is the average proportion of damaged leaves per treatment in a given week. Standard errors are not shown to facilitate the visualization of the trends of each treatment. The data from the 6th to the 9th weeks are based on samples of 30 leaves per cage. Different letters in the legend represent significant differences among treatments for these weeks (contrasts with general linear hypothesis testing after LME,  $P < 0.05$ ). The data of the 10th week (right of the dashed line) are based on destructive sampling. Treatments: Control: thrips alone; *T. putrescentiae*: thrips + alternative prey; *A. swirskii*: thrips + predatory mites; *A. swirskii* + *T. putrescentiae*: thrips + predators + alternative prey. **(b)** The average ( $\pm$  s.e.) number of thrips on leaves (light gray) and in flowers and buds (dark gray) in the 10th week. Different letters inside the bars indicate significant differences among treatments (contrasts with general linear hypothesis testing after GLM,  $P < 0.05$ ).