Biological Control 95 (2016) 5–12

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Non-consumptive predator effects on a primary greenhouse pest: Predatory mite harassment reduces western flower thrips abundance and plant damage

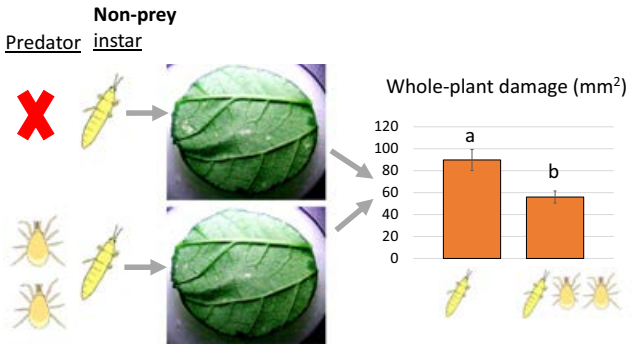
S.E. Jandricic *, Danielle Schmidt, G. Bryant, S.D. Frank

Department of Entomology, Gardner Hall, NC State University, Raleigh, NC 27607, USA

HIGHLIGHTS

- Non-consumptive effects of predatory mites on non-prey stages of thrips were studied.
- *Neoseiulus cucumeris* reduced feeding activity of 2nd instar western flower thrips by 22%.
- Whole plant damage from 2nd instar thrips was reduced 38% when mites were present.
- Survival of 2nd instar thrips was also significantly reduced when mites present.
- "Harassment" of thrips by mites likely aids in thrips control in greenhouse crops.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 June 2015
Revised 21 December 2015
Accepted 22 December 2015
Available online 23 December 2015

Keywords:
Non-consumptive predator effects
NCEs
Trait mediated interactions
Trophic cascades
Frankliniella occidentalis
Neoseiulus cucumeris

ABSTRACT

Prey react to the presence of predators in suite of ways that reduce predation risk, but may also negatively affect fitness. Non-consumptive effects (NCEs) of predators on prey are likely important components of biological control and moderators of plant damage in agricultural systems. Yet, few studies have investigated their effects in crops relying on augmentative release of natural enemies for protection. Here, we investigated NCEs of the predatory mite *Neoseiulus cucumeris* on a non-prey life stage of western flower thrips (*Frankliniella occidentalis*), one of the most damaging pest of greenhouse crops in the world. Second instar thrips were exposed to 2 adult female mites on a leaf disk for 24 h. Over a 20 min observation period, we saw a 22% reduction in thrips feeding behavior in the presence of predatory mites compared to thrips alone. Thrips feeding was often interrupted by attempted mite attacks, which averaged 4 attacks per thrips over 20 min. After 24 h, this reduced leaf damage by 51% in the predator treatment compared to the control. This result held true in experiments on whole plants, with damage reduced by 38% in the presence of mites. No significant reduction in feeding activity or damage was observed when larval thrips were exposed to non-predatory mites, conspecifics, or leaves which had previously held large numbers of predatory mites. The presence of mites did not alter thrips development time or final adult size. However, survival to adulthood was decreased by 54–78% in the presence of mites, suggesting a lack of nutritional reserves necessary to complete development. This study demonstrates that NCEs of predatory mites can induce a trophic cascade by reducing pest feeding and fitness. Such beneficial effects of natural enemies are often overlooked in simple predation and efficacy studies.

* Corresponding author at: Ontario Ministry of Agriculture and Rural Affairs, 4890 Victoria Ave. North, Vineland Station, ON L0R 2E0, Canada.
E-mail address: sarah.jandricic@ontario.ca (S.E. Jandricic).

http://dx.doi.org/10.1016/j.biocontrol.2015.12.012
1049-9644/

1. Introduction

In the field of biological control, efficacy of a particular candidate predator is generally measured by a combination of prey consumption rate and reproductive capacity on the prey item (see such studies as McGregor et al., 1999; Aruthurs et al., 2009; van Maanen et al., 2010). However, these are hardly the only effects worth consideration. Just the presence of predators, or their chemical cues, may significantly alter life history traits or the behavior of a prey species, even in the absence of density-dependent effects like consumption (Werner and Peacor, 2003). These are termed "trait-mediated interactions" at the population level, or "non-consumptive effects" (NCEs) at the individual level. NCEs of predators can lead to defensive prey adaptations such as changes in foraging behavior (e.g. Schmitz et al., 1997; Morrison, 1999; Rypstra and Buddle, 2013), oviposition strategies (e.g. Grostal and Dicke, 1999; Fernandez-Ferrari and Schausberger, 2013), and even morphology (e.g. Boersma et al., 1998; Relyea, 2003), all designed to reduce predation risk of the prey.

NCEs are of importance to biological control because producing such phenotypic changes to avoid predators can be costly for prey. Ecological studies illustrate that prey commonly have reduced fitness traits such as longer development, lower fecundity, and smaller body size in the presence of predators (e.g. Barry, 1994; Fischer et al., 2012; Peckarsky et al., 2002). Ultimately, these fitness tradeoffs can play a significant role in limiting prey populations. In a recent meta-analysis by Preisser et al. (2005), NCEs of predators were shown to have equal-or-greater effects on prey population size than direct consumption. Preisser et al. (2005) also demonstrated that NCEs seem more likely than consumptive effects to have strong carry-through effects across trophic levels, likely due to effects on both prey density and foraging behavior (Preisser et al., 2005). The potential ability of NCEs to cause trophic cascades – *i.e.* indirect effects on plants by predators, mediated by herbivores (Schmitz et al., 2004) – is especially relevant to biological control in agricultural and horticultural crops, since the ultimate goal is decreasing plant damage, rather than decreasing herbivore abundance *per se*.

The bulk of studies on NCEs to date have been done in natural ecosystems (see Preisser et al., 2005). However, recent examples suggest that predator NCEs have a stronger role in agricultural arthropod pest control than previously thought. For example, Costamagna et al. (2013) demonstrated that predation by lady beetles on top leaves of plants forced surviving aphids to use less suitable feeding sites lower on the plant, reducing aphid population growth by 27–130% compared to predator-free aphid colonies. Nelson et al. (2004) found that damsel bugs (Hemiptera: Nabidae) with amputated mouth parts reduced pea aphid population growth by 30% on alfalfa plants. But, as pointed out by Walzer and Schausberger (2009), studies of NCEs in systems extensively relying on augmentative biological control – *i.e.* greenhouse crops – are limited. This is surprising, given that one could hypothesize that effects of NCEs are likely more evident in "closed" systems where natural enemies are released at high rates. Existing studies in augmentative biocontrol systems are mostly confined to one pest – spider mites – and their predators. These studies generally show reduced spider mite oviposition or changes in oviposition sites due to NCEs (e.g. Choh et al., 2010; Lemos et al., 2010; Walzer and Schausberger, 2011), but increased dispersal (Fernandez-Ferrari and Schausberger, 2013) or effects on growth (Bowler et al., 2012) have also been demonstrated. The handful of studies on NCEs in augmentative biocontrol outside of the spider mite system have demonstrated reduced insect settling on plants (Lee et al., 2011, 2014; Wong and Frank, 2012), and decreased pest feeding, survival and oviposition (Walzer and Schausberger, 2009). More studies on the power of NCEs are needed on other important greenhouse pests, such as thrips, aphids, whitefly, fungus gnats and lepidopteran pests.

Given that greenhouse crops often consist of monocultures, predator–prey interactions are likely to be stronger and have greater effects on basal resources. Thus, it is also surprising that studies on trophic cascades resulting from NCEs are virtually non-existent within augmentative biocontrol systems. To date, only Skaloudova et al. (2007) and Walzer and Schausberger (2009) have demonstrated that plant biomass can be affected by NCEs on greenhouse pests. Unfortunately, the value of these studies is somewhat limited, since they were conducted on excised leaves, and their results are in opposition (with Skaloudova et al. (2007) showing increased pest plant damage in the presence of predator NCEs, but Walzer and Schausberger (2009) demonstrating reduced damage). More studies on the ability of NCEs to result in trophic cascades are needed; especially studies on whole plants, where greater avoidance of predators is possible. Only by identifying all known predator effects and assessing these outside of the Petri dish can we comprehensively understand factors affecting pest abundance and plant damage, and capitalize on these to improve control of difficult pests.

To add to this knowledge, we study NCEs of the predatory mite *Neoseiulus cucumeris* Oudemans. *N. cucumeris* is one of the main predators used in the biological control of western flower thrips (*Frankliniella occidentalis* Pergande; Thysanoptera: Thripidae), oft considered the most serious pest of greenhouse crops in the world (Wardlow, 1989; Fery and Schalk, 1991). This mite is commercially available, relatively inexpensive (ca. 1 cent for 10 mites, or $50 USD per 50,000 mites, including shipping costs; J. Maurer, Association of Natural Biocontrol Producers, personal communication), and generally effective (e.g. Gillespie, 1999; Jacobson et al., 2001; Shipp and Wang, 2003). *N. cucumeris* are able to consume 1st instar larvae of *F. occidentalis* (reported averages vary from 1 per day to 6 per day; Shipp and Whitfield, 1991; Buitenhuis et al., 2010), but other thrips life stages are not susceptible to predation due to their larger size (van der Hoeven and van Rijn, 1990; Shipp and Whitfield, 1991; Shipp and Wang, 2003). In the only published study on NCEs of mites on thrips, Walzer and Schausberger (2009) demonstrated that the presence of even a non-predaceous stage of a predator (*i.e.* eggs of *Neoseiulus californicus*) can reduce feeding time, survival and leaf damage from 1st instar *F. occidentalis*. Similarly, we predicted that adult *N. cucumeris*, despite only feeding on 1st instar thrips, may reduce feeding time, development, survival and leaf damage of later thrips instars, especially if the mites repeatedly attempt to attack 2nd instar thrips. Further, we advance research of NCEs of predatory mites by investigating their ability to cause trophic cascades on whole plants.

2. Materials and methods

2.1. Source and maintenance of arthropods

F. occidentalis of all stages were obtained from a lab-reared colony maintained at Cornell University (Ithaca, NY) on cranberry bean (*Phaseolus vulgaris*). Insects were transferred and maintained on red kidney bean (also *P. vulgaris*) var. "Pink Panther" (Harris Teeter, Charlotte NC) and reared in a growth chamber at 27 ± 1 °C, 50–60% RH, and 16:8 L:D. To obtain 2nd instar larvae (henceforth referred to as L2s), 25–30 adult *F. occidentalis* of unknown age were transferred from the main colony using an aspirator, made from a modified centrifuge tube, and placed in "cohort containers" comprised of Sterilite Ultra-Seal 3.8L plastic containers (Sterilite Corporation, Townsend MA). For ventilation, each container had a 9 cm^2 hole cut in the lid, plus 9 additional 3 mm diam-

eter holes drilled in a rectangular pattern in all four sides; all holes were covered with thrips-proof screening. Within each container we placed 2 bean seedlings as a food source and oviposition site for the thrips. Bee pollen was lightly dusted on the top of the leaves as a protein source. Adult thrips were allowed to oviposit for 24 h and then removed from plants. After 7 d, >50 L2s were available per container for use in experiments.

All stages of predatory mites (*N. cucumeris* (Oudemans)) were obtained from Applied Bionomics (Victoria, BC, Canada) in the form of "breeder packs" – envelopes filled with bran and cereal mites (*Tyrophagus putrescentiae* Schrank) as a food source for *N. cucumeris*. Adult female mites of unknown age were selected directly from these packs for use in experiments, as were adult females of *T. putrescentiae* in Section 2.3.

2.2. Plant material

Red kidney beans (*P. vulgaris*) var. "Pink Panther" were used for all experiments. Seeds were germinated in seedling trays in 2B Mix (Fafard®, Anderson, SC) and grown in a growth chamber (27 ± 1 °C, 50–60% RH, 16:8 L:D) until use in experiments. Plants were watered 3× per week with fertilizer solution (Peter's Professional Fertilizer, 20:10:20; Everris International, BV, The Netherlands) at 150 ppm. For whole-plant experiments (Section 2.5), plants were ca. 14 d old, planted in 10-cm pots, and had at least 2 true leaves.

2.3. Effects of predatory and non-predatory arthropods on L2 thrips feeding behavior and leaf damage

L2 thrips were obtained as in Section 2.1, and were 4 d ± 1 d post-eclosion at the time of experiments. Tests to determine effects of predatory mites on L2 thrips were conducted in 30 mL plastic deli containers (7 cm diameter opening; Dart Container Corporation, Mason, MI). Two cotton pads (4 cm in diameter; U.S. Cotton, Castonia, NC) were placed in the bottom of each cup, which was filled with 50 mL of tap water. A 4 cm diameter leaf disc (cut using a sharpened metal pipe) was then "floated" on the water-soaked cotton pads. This kept the leaf discs hydrated while also confining the insects to the discs. One L2 thrips was added to each leaf disc using a fine paint brush. Thrips were then immediately exposed to either (i) a no-treatment control or (ii) 2 adult female *N. cucumeris*. Adult female predatory mites were chosen because they are the only mite life stage that is considered to effectively reduce thrips populations (Shipp and Whitfield, 1991). Mites were also added with a fine paintbrush. After allowing 2 h for all insects to settle, the behavior of each thrips was observed under a dissecting microscope (6.7 to 20× magnification) for a continuous 20 min. Behaviors were scored as either feeding or non-feeding and were timed using digital stopwatches. Feeding was confirmed by the presence of (i) "bobbing" head movements and (ii) movement of abdominal contents as seen through the integument. Non-feeding included both "resting" (when the thrips was stationary, but no evidence of feeding took place) and "walking" (which included all ambulatory movements, except for repositioning when probing/feeding). Proportion of time spent feeding is presented.

After the observation period, cups were covered with thrips-proof screening, which was secured with the container lid. A 1 cm^2 hole was cut in the center of each lid for ventilation. Cups were held in an incubator at 25 °C and 16:8 L:D cycle. After 24 h, leaf discs were removed, and the leaf area damaged by thrips feeding (*i.e.* "silvering") was measured under a microscope. This was done by overlaying the leaf with a screen with 1 mm^2 openings, and counting the number of squares with damaged tissue. The number of thrips that died over the 24 h period (including cadavers found on the leaf disc or in the water moat) were also recorded and reported. This trial was repeated twice (on different dates, with new batches of thrips), with 15 replicates per treatment in each trial replicate.

To confirm that behaviors were elicited by NCEs, and not merely by the presence of other arthropods on the leaf, the entire trial was repeated with the addition of two non-predatory arthropod treatments. The 4 treatments were as follows: (i) no-treatment, (ii) 2 adult female *N. cucumeris* (iii) 2 adult female cereal mites (supplied in sachets of *N. cucumeris* as food for this predator), or (iv) 2 conspecific L2 thrips. Arthropods in treatments ii–iv were added directly to the leaf disc with a fine paintbrush. Adult female cereal mites are of similar size as *N. cucumeris* adults. Methods were identical to the experiment above, with the following exceptions. In the treatment with multiple thrips, the first L2 encountered randomly under the microscope was chosen as the "test" thrips. For those in the "conspecific" treatment, the final area damaged was divided by 3 to obtain an estimate of damage per individual L2 thrips. Along with activity, encounter rate was also measured – by recording the number of times the test thrips was physically touched by another arthropod in the arena – as was the number of defensive responses exhibited by the L2. Defensive behavior consisted of abdominal "jerking", as described in Bakker and Sabelis (1989), the production of a drop of rectal fluid, or walking away from the stimulus. This trial with additional treatments (and measures of encounter rates) was repeated twice, with 9–15 replicates per treatment for each trial replicate.

For ease of presentation of results (and because trends were similar across experiments), data from the above trials were combined for analyses (see Section 2.7). In all trials, 24 h damage data for a particular replicate (cup) were omitted from analysis if (i) the thrips was found dead, (ii) if it was evident that the thrips had not fed at all in 24 h (indicating that their mouthparts may have been damaged during transfer), or (iii) if either of the two mites or conspecifics had died (usually a result of drowning in the water moat), since this would represent a loss of stimulus in somewhere in the 24 h period, and could lead to an underestimate of predator effects. Most loses occurred in replicates with mites, which seemed more prone to walk off the leaf and drown. Thus, our final number of replicates analyzed for damage was $n = 15$ in the cereal mite treatment (down from $n = 20$ in the observation portion of the experiment), $n = 31$ in the predator treatment (down from 45), $n = 21$ in the conspecific treatment (down from 23), and $n = 44$ for the controls (down from 51 replicates in the observation portion of the experiment).

2.4. Effects of chemical "footprints" of predators on L2 thrips feeding behavior and leaf damage

We also conducted an experiment to determine if changes in behavior of thrips larvae could be caused by chemical "footprints" from predators (*i.e.* cues such as cuticular secretions, feces, and other excreta) potentially left behind on floating leaf discs. Methodology was similar to experiments described in Section 2.3, with the following exceptions. Treatments consisted of a "low" (4 adult female mites/leaf) and a "high" (12 adult females mites/leaf) predator treatment, as well as a control (no mites). Mites occupied leaves for a minimum of 3 h, but were removed immediately prior to the addition of the L2 thrips. No mites were lost during this 3 h interval. Thrips were only allowed to acclimatize for 20 min before observation (instead of 2 h), so that any potential volatiles from the mites did not have a chance to dissipate. Thrips behavior and 24 h leaf damage were assessed as before. The trial was conducted 3 separate times for a total of 24 replicates per treatment. As in Section 2.3, data were omitted from the damage analysis if the thrips could not be recovered after 24 h.

2.5. Effects of mites on thrips mortality and development time

Thrips cohorts were set up as in Section 2.1 in a growth chamber set at 25 ± 1 °C and 50–60% RH. Bean seedlings were observed 3× a day for egg hatch. When eclusion of L1 thrips was noted, the experiment was initiated 24 h later by transferring individual L1 thrips to leaf discs in the arenas described in Section 2.3. Thus, all thrips were 12 ± 12 h old on Day 0 of the experiment. Thrips were observed daily for changes in development. On Day 3 of the experiment, all L1s had reached L2 stage, as evidenced by the size of the head capsule relative to body size. At this point, cups received 1 of 3 treatments: 2 adult female *N. cucumeris* mites, 4 adult female *N. cucumeris* mites, or no mites (control). Since a study by Bakker and Sabelis (1989) demonstrated that *N. cucumeris* were able to successfully kill a small proportion of 2nd instar *Thrips tabaci* when starved for long periods, we replaced the predatory mites with new mites every 48 h as an extra precaution (though it should be noted that we could find no publications indicating *N. cucumeris* can successfully prey on 2nd instar *F. occidentalis*, and that *T. tabaci* is a smaller thrips species; van Rijn, 1995). On Day 5, we began observing thrips 3×/day to provide more accurate estimates of time to pupation and time to adult emergence. Length of pupal and adult thrips was measured using an ocular ruler, and adults were sexed at the end of the experiment. In all cases, thrips (and mites, where appropriate) were transferred to fresh leaf discs every 48–72 h.

The trial described above was repeated twice. There were 30 replicates per treatment in the first trial. In the second, we decreased the control replicates to 25, while increasing the replicates of the low-density mite treatment to 75. This was done to compensate for the significant mortality in the mite treatments (see Section 3) and give us a large enough sample size of adult thrips in each treatment at the end of the experiment to make statistical comparisons regarding development time and body size. We omitted the "high" mite treatment in the second replicate since few thrips survived this treatment. The number of replicates needed in the high mite treatment to provide a reasonable number of data points at the end of the experiment would have been logistically unfeasible.

2.6. Effect of mites on thrips damage on small plants

To determine if leaf damage effects seen in Section 2.3 would scale up, we conducted a similar experiment on small, whole plants. Plant material and selection of thrips is described in Sections 2.1 and 2.2. To a randomly-selected cotyledon leaf on each plant, 8 L2 thrips were added to the adaxial side using a fine paintbrush. To half of these plants, 16 adult female *N. cucumeris* were added immediately after the thrips to the same leaf (providing the same predator–prey ratio as in the leaf-disc experiments). There were 19 replicates of each treatment (predators present; predators absent). All arthropods were left on the plants, and allowed to distribute naturally, for 48 h in a room with natural light, held at a constant 24 °C and 50% RH. Plants were inspected with a magnifying visor at 24 h to make sure mites could still be found in the predator treatment. At the end of the experiment, plants were destructively sampled, and the abaxial and adaxial sides of all leaves were scanned at 24-bit color and 600 dpi on a scanner (Epson Perfection V500). From these images, amount of damage per leaf (converted to mm^2) was determined at a later date using image-processing software (ImageJ).

2.7. Statistics

The effect of predator or arthropod treatment was assessed using a mixed-model ANOVA in SAS (v. 9.3; SAS Institute, 2011). Activity data (expressed as a proportion of time spent feeding) were arcsine transformed, and damage data (expressed in mm^2) were log $x + 1$ transformed to better meet assumptions of ANOVA. Although the heterogeneity of errors was addressed using transformations, but normality was generally not, we also included the non-parametric Kruskal–Wallis test for all data. If the non-parametric results generally agreed with the parametic test, the results of the ANOVA are presented. Since all comparisons within the ANOVAs were planned, Fisher's protected LSD test was used to compare multiple means. In all cases, trial replicate and treatment replicate (cup; since later replicates were observed for behavior later in the day) were included as random effects; treatment replicate was nested within trial. If trial contributed $\leqslant$30% to the variance component of the analysis, we considered it acceptable to combine trials, especially since trends were consistent (although it should be noted that this threshold was an arbitrary cut-off). Cases where effect of trial exceeded 30% of the variance component are reported in the Results section. Treatment replicate contributed less than 18% to the variance component in all cases, and is not reported. Where encounters and defensive responses to mites or conspecifics were observed (Section 2.3), arthropod species was the fixed effect and random effects were as above.

Data from the 3× daily observations of thrips (Section 2.5) were used in calculations of development time. The midpoint of each observation interval was used, as in Jandricic et al. (2010). Developmental time data, an example of time-to-event data (whose distributions are commonly skewed to the right), were ln $(x + 1)$ transformed. L1-to-adult development time, pupal size and adult size were investigated in a series of mixed-model ANOVAs, where trial replicate was the random effect. Treatment, sex of the thrips, and their interactions were the main effects. The proportion of thrips surviving to adulthood in each treatment was analyzed using a logistic regression in Proc Glimmix in SAS, specifying a binomial distribution.

Additionally, we investigated thrips mortality before mites were removed (at the pre-pupal thrips stage) and after, to determine if there were any continuing NCEs once the predators were removed. We used a logistic regression, with trial replicate as a random effect and treatment as the main effect. Results of Type 3 tests of fixed effects are reported. As for all other tests, arithmetic means and standard errors are presented, though analyses were performed on transformed data.

3. Results

3.1. Effects of predatory and non-predatory arthropods on L2 thrips feeding behavior and leaf damage

Average percent mortality of thrips in all treatments and trials was <17% over the 24 h period (this included "missing" thrips that were assumed dead). A logistic regression detected no significant effect of treatment on the number of dead thrips ($F_{(3,183)} = 0.72$, $P = 0.54$). Thrips mortality in the predatory mite treatment was 14 ± 4.4% over 24 h, which was similar to the mortality in our control treatment (10 ± 3.7%). This confirms that *N. cucumeris* adult mites are unable to kill L2 thrips, and that predatory mites did not cause an increased escape response in thrips (which would be expected to lead to higher mortality in predator cups due to drowning in the moat).

The ANOVAs on transformed data revealed that arthropod treatment significantly affected the amount of time L2 thrips spent feeding ($F_{3,96.7} = 3.63$, $P = 0.0156$), and the amount of damage per leaf over 24 h ($F_{3,67.2} = 8.86$, $P \leqslant 0.0001$). Using graphical assessment, all assumptions of ANOVA for these analyses were met after data transformation, except for normality (Shapiro–Wilk tests:

$W \geqslant 0.94$, $P \leqslant 0.011$). However, the Kruskal–Wallis test (which does not depend on normality), was marginally significant for time spent feeding ($\chi^2_{(3)} = 6.89$, $P = 0.07$) and significant for damage ($\chi^2_{(3)} = 19.74$, $P = 0.0002$). Thus, we chose to accept and present the results of the parametric ANOVAs.

Thrips spent 22% less time feeding with predatory mites present, compared to the controls ($t_{88.4} = 2.93$, $P = 0.0043$; Fig. 1A), and plant damage was reduced by 51% compared to the control ($t_{129} = 4.57$, $P \leqslant 0.0001$; Fig. 1B). Proportion of time spent feeding and leaf damage were not affected by conspecific thrips or cereal mites compared to the controls ($t_{98.7} \leqslant 0.62$ and $P \geqslant 0.5$ and $t_{76.1} \leqslant 1.16$ and $P \geqslant 0.25$, respectively). Comparing predatory and non-predatory mites, *N. cucumeris* reduced thrips feeding time by 18% compared to *T. putrescentiae*, though this reduction was not statistically significant ($t_{108} = 1.55$, $P = 0.12$; Fig. 1A). Damage was 46% lower with predatory mites vs. non-predatory mites, which was significant ($t_{79.8} = 2.14 = 0.036$; Fig. 1B).

Arthropod species also had a significant effect on encounter rate ($F_{2,59} = 14.55$, $P < 0.0001$ for the parametric ANOVA; $\chi^2_2 = 24.5$, $P \leqslant 0.0001$ for the Kruskal–Wallis test). The thrips under observation was significantly more likely to be attacked by a predatory mite than have an encounter with a cereal mite or conspecific thrips (Fig. 1C; $t_{59} \geqslant 3.37$, $P \leqslant 0.004$ for all *t*-tests). There was an average of 3.9 ± 0.76 attacks per thrips by *N. cucumeris* occurring over 20 min (range: 0 to 14). This was 55% higher than the number of encounters with conspecifics, which only seemed to interact when they were fighting over a feeding site. Results of a logistic regression indicated that whether a thrips engaged in defensive behavior was significantly predicted by arthropod species ($F_{1,59} = 8.44$, $P = 0.0006$). Thrips exhibited a significantly higher number of defensive reactions (*i.e.* wagging or walking away) in response to predatory mites than other treatments (Fig. 1C; $t_{59} \geqslant 3.00$, $P \leqslant 0.0039$), with over 2.5 reactions in 20 min.

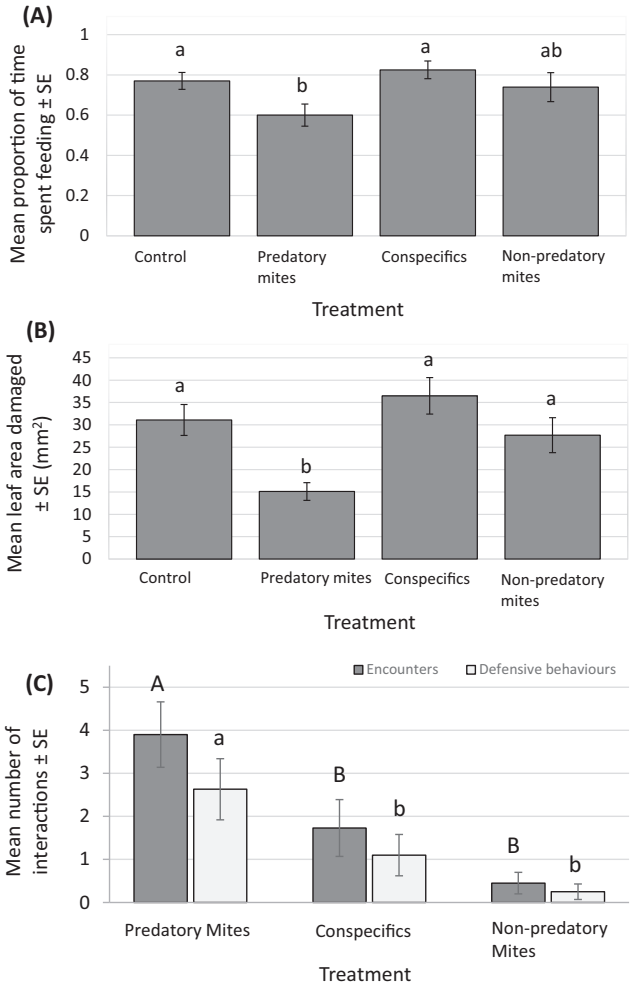


Fig. 1. (A) Mean proportion of time spent feeding (±SE) by an individual 2nd instar Western flower thrips when alone, or in the presence of 2 predatory mites (*Neoseiulus cucumeris*), 2 conspecific thrips, or 2 non-predatory mites (*Tyrophagus putrescentiae*). (B) Mean leaf area damaged (mm^2 ± SE) by the same set of thrips over 24 h. Total area of the leaf disc was 159 mm^2; only one side of the leaf was available to the thrips. (C) Mean number of encounters by the observed thrips with other arthropods and mean number of defensive responses these elicited. In all cases, different letters indicate significant difference ($\alpha = 0.05$).

3.2. Effects of predator chemical "footprints" on L2 thrips feeding behavior and leaf damage

In the experiment assessing effects of potential chemical mite residues on thrips, there was no effect of treatment on time spent feeding or amount of feeding damage (Fig. 2; $F_{2,43.7} = 0.82$, $P = 0.45$ and $F_{2,50} = 1.63$, $P = 0.21$, respectively) using the parametric ANOVAs. Results of the non-parametric Kruskal–Wallis tests concurred ($\chi^2_2 = 1.58$, $P = 0.45$ and $\chi^2_2 = 1.07$, $P = 0.58$, respectively). In the mixed-model analysis of damage, we had a large effect of trial (60% of the variance component). Thus, trials were also assessed separately. Though the amount of total damage varied between trials, treatment did not have a significant effect on leaf damage caused by thrips in any trial replicate ($P \geqslant 0.20$), confirming our overall analysis.

3.3. Effects of mites on thrips mortality and development time

Survival to adulthood varied significantly between the treatments ($F_{2,153} = 7.73$, $P = 0.0006$; Fig. 3). Survival was significantly higher in the control compared to both mite treatments ($t_{153} \geqslant 2.96$, $P \leqslant 0.0036$). There was no difference between survival

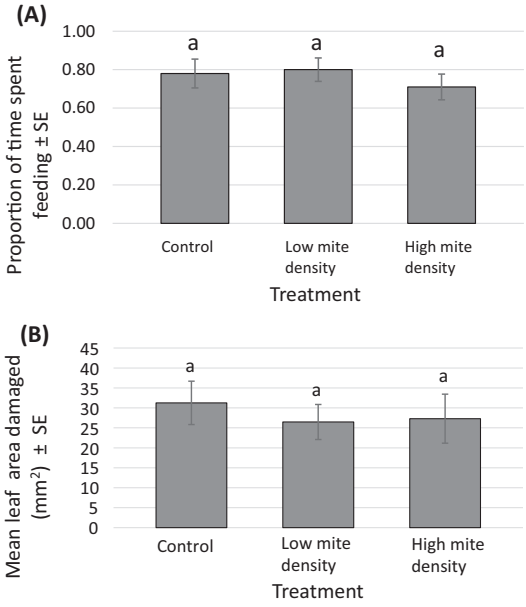


Fig. 2. (A) Mean proportion of time spent feeding (±SE) by individual 2nd instar Western flower thrips on leaves potentially containing chemical "footprints" of no mites (control), 4 adult *N. cucumeris* (low mite density), or 12 adult *N. cucumeris* (high mite density). Mites resided on leaves for 2–3 h prior to the addition of the thrips. (B) Mean leaf area damaged (mm^2 ± SE) by the same set of thrips over 24 h. Total area of the leaf disc was 159 mm^2. In all cases, different letters indicate significant difference ($\alpha = 0.05$).

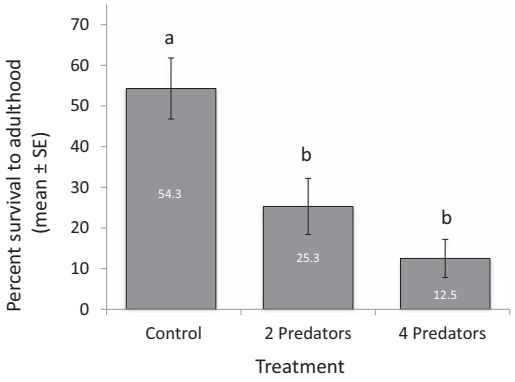


Fig. 3. Percent survival (±SE) to adulthood of Western flower thrips in the presence of no predatory mites (control), or 2 or 4 adult female *Neoseiulus cucumeris*. Mites were added when thrips were too big for mites to consume (L2 stage), and were replaced every 48 h to prevent starvation from increasing the likelihood of successful mite attack. Different letters indicate significant differences ($\alpha = 0.05$).

rates in the low and high mite treatments ($t_{153} = 1.70$, $P = 0.09$). Much of the mortality was incurred before thrips started to pupate. In the control treatment, 47% of the total mortality occurred in the 2nd instar larval stage. In the mite treatments, 73–81% of the total mortality occurred in the 2nd instar larval stage *(i.e.* when mites were present).

We also took the thrips that survived to pre-pupation (*i.e.* when mites were removed) and analyzed their mortality separately, with this representing long-lasting effects from previous NCEs. This was only done for the control and low-density mite treatments, as there were not enough replicates to include the high-mite treatment. We observed 31 ± 7.8% mortality of thrips after the pre-pupal stage in the control group; this was arithmetically lower than the 49 ± 7.9% mortality observed in the mite-exposed treatment. However, this result was not statistically significant ($F_{1,74} = 2.61$, $P = 0.11$).

For those thrips that survived to adulthood, there was no significant difference in L1-to-adult development time in the presence or absence of mites (9.2 ± 0.18 vs. 9.2 ± 0.17 days, respectively; $F_{1,45} = 0.01$, $P = 0.98$), even when only female thrips were analyzed ($F_{1,26} = 0.01$, $P = 0.94$). As expected, female thrips were significantly larger than males ($F_{1,38} = 20.65$, $P \leqslant 0.0001$). Females were not significantly different in size in the presence or absence of mites (vs. 1.81 ± 0.04 versus 1.86 ± 0.05 mm long, respectively; $F_{1,27} = 0.01$, $P = 0.99$).

3.4. Effect of mites on thrips damage on small plants

Trends in our experiment on small plants were similar to those on leaf discs. Treatment significantly affected plant damage ($F_{1,35} = 7.85$, $P = 0.008$ for the parametric analysis; $\chi^2_1 = 8.15$, $P = 0.0043$ for the Kruskal–Wallis test; Fig. 4), with mites reducing total leaf area damaged by 37% compared to the control. There was no effect of treatment on number of leaves damaged or the number of damage spots per plant ($F_{1,35} \leqslant 1.76$, $P \geqslant 0.19$ for all tests).

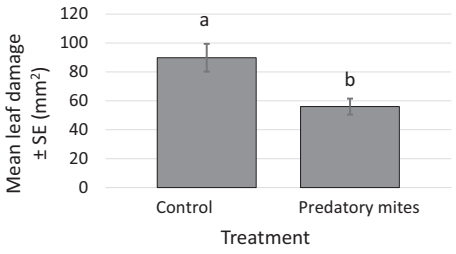


Fig. 4. Mean total leaf area damaged on whole small bean plants (mm^2 ± SE) from the addition of 8–2nd instar Western flower thrips alone, or in the presence of 16 adult predatory mites (*Neoseiulus cucumeris*) after 48 h. Different letters indicate significant difference ($\alpha = 0.05$).

4. Discussion

Our study demonstrates that non-consumptive effects of adult predatory mites can reduce larval thrips feeding, whereas non-predatory arthropods do not. Ultimately, NCEs from predatory mites reduced thrips abundance by negatively affecting larval thrips survival, and improved plant quality by decreasing thrips feeding damage. Results were similar between leaf disc and whole plant experiments, demonstrating real-world applications to horticultural crop production. This is especially important for growers of ornamental crops where consumer tolerance for pest damage is low.

Though our observational studies revealed some decrease in thrips feeding behavior in the presence of predators, leaf damage over 24 h is more indicative of NCEs on plants over time. On leaf-discs, we demonstrated that presence of predatory mites can cause a trophic cascade, reducing leaf damage from thrips by 50%. This was less than the reduction in damage found by Walzer and Schausberger (2009) with 1st instar thrips in the presence of mite eggs, where a 77% reduction in damage was observed. Oviposition allomones from predator eggs may be perceived as a more constant predation threat to 1st instar thrips compared to sporadic attacks by adult mites on 2nd instars. Or, their smaller experimental arena may have concentrated effects. Regardless, ours is the only study with thrips to scale up investigations of NCEs from predators onto a whole-plant scale. We find it positive for pest management that, even when larval thrips could move to various sites on the plant, the strength of NCEs were similar to those in the lab, with a 37% reduction in plant damage on whole bean plants.

The reduction in thrips feeding damage by 37–50% in the presence of predators in our trials is similar to previous studies of NCEs on agriculturally important terrestrial arthropods (though numbers of these studies are limited). For example, Thaler and Griffin (2008) found a 33% reduction in damage caused by *Manduca sexta* (Lepidoptera: Sphingidae) in the presence of a predator (*Podisus maculiventris* (Heteroptera: Pentatomidae)) with its mouthparts removed. Rypstra and Buddle (2013) found that scarab and coccinellid beetles caused 50% less plant damage when spider webbing was present. Thus, the strength of effects of NCEs on pest insect herbivory rates seems fairly consistent across insect orders, though more work is needed.

Despite similar outcomes from NCEs across studies, mechanisms behind the results likely differ between the organisms and life stages involved. Several studies suggest that chemical cues from predatory mites contribute to herbivorous mites. For example, in no-choice trials, Fernandez-Ferrari and Schausberger (2013) found delayed and reduced oviposition by spider mites (*Tetranychus urticae*) in the presence of various predator mite chemicals. Grostal and Dicke (1999) and Fernandez-Ferrari and Schausberger (2013) both found that when given a choice, spider mites avoided leaves with chemical cues from certain predatory mites. We found no effect of adult predatory mite chemicals on *F. occidentalis*, even when a high density of mites were left on leaves for several hours. Since we did not conduct choice trials, however, we can't rule out that thrips may have chosen to feed on mite-free leaves versus mite-exposed leaves. But, since we saw no increase in thrips activity on mite-exposed leaves in our no-choice trials, this seems unlikely. Though previous studies con-

firm that thrips can use olfactory cues to find host plants (Van tol et al., 2012; Teulon et al., 1993) or to avoid or destroy predator eggs (Walzer and Schausberger, 2009), there are no studies demonstrating that thrips react to chemical cues left by larval or adult predators of any kind (which may be more dilute than pheromones left on eggs). However, it's possible we may have seen different results if the predatory mites were first allowed to feed on thrips, instead of astigmatid mites, as predator diet can effect anti-predator behavior in arthropods (Mendel and Schausberger, 2011).

Predatory mites used in greenhouses consume thrips, but only when the thrips are in in the L1 stage, which lasts for a relatively small window of time (ca. 2 d). For L1 thrips that escape predation and continue to develop, our results show that mites still affect their abundance via NCEs. When L2 thrips were continually exposed to two adult mites, 53% fewer thrips survived to adulthood. Survival to adulthood was reduced by 77% when 4 predators per leaf were present. This dramatic effect on late-instar larval survival likely accounts for a sizable amount of thrips population reduction in greenhouses. One caveat to our studies is that the preferred prey of *N. cucumeris*, L1 thrips, were absent. However, in the greenhouse, where thrips have overlapping generations, mites are likely to encounter every thrips life stage while foraging. Further, our study provides a representation of what likely occurs at augmentative release rates of predatory mites in greenhouses once most of the easily available prey are consumed.

The reduced larval thrips survival in our study is most likely from reduced nutrition and/or stress-induced effects on the thrips endocrine system. When under attack, thrips would either walk away and attempt to find a new feeding site, or cease feeding and repeatedly jerk their abdomen at the attacking mite. Thrips exposed to predators in our experiment either displayed jerking or walking over 2.5 times in a 20-min interval, which would translate to more than 180 interruptions in feeding in 24 h. Though previous studies have described abdominal jerking as thrips defensive behavior (e.g. Bakker and Sabelis, 1989), we further suggest that this action has important energetic costs for thrips that contributed to the reduced survival of larvae. Even after mites were removed in our study, pre-pupal thrips that were exposed to mites as larva were 20% more likely to die before reaching adulthood than those in the control treatment. This suggests reduced nutritional reserves in larval thrips – or possibly an upset hormonal system – resulted in a proportion of pupae too weak to complete development. This could be an important finding in terms of pest control, as weakened pre-pupae, which fall to the soil to pupate, should theoretically be more susceptible to co-applications of entomopathogenic fungi or nematodes due to reduced immune capacity, as in Ramirez and Synder (2009). However, factorial studies including foliar predatory mites and nematodes have not necessarily shown better thrips control when both are used (Ebssa et al., 2006; Pozzebon and Boaria, 2014; Jandricic et al., unpublished data). Further investigation is needed.

Our study is the first to demonstrate that L2 thrips can be "intimidated" by adult predators, even when no real threat exists, and that these NCEs provide real reductions in thrips abundance and damage. Walzer and Schausberger (2009) previously demonstrated that adult thrips oviposition can be reduced in presence of predatory mite eggs. Together, these studies illustrate that prey life stages non-susceptible to predation by a predator can still serve as important targets for biological control via NCEs. These data also demonstrate that the same predator is capable of affecting the behavior and fitness of prey via NCEs across multiple prey (and predator) life stages, though the mechanisms may differ ontogenically. Over the lifetime of both the prey and the predator, these affects likely add up to an even larger contribution by NCEs than these individual studies have captured. Thus, these joint findings suggest that, rather than biological control occurring exclusively through predation on L1s, it is likely that mite NCEs on other thrips stages also play an important role in reducing thrips abundance and crop damage.

Acknowledgments

Thanks to Nicole Bissonnette and Laura Daly for their technical help. Thank you to Dr. Rebecca Loughner (Cornell University) for advice and the source of thrips. Funding provided in part by grants from American Floral Endowment and Gloekner Foundation to SDF. Predatory mites were generously donated by Applied Bio-nomics Ltd (Canada).

References

Aruthurs, S., McKenzie, C.L., Chen, J., Dogramaci, M., Brennan, M., Houben, K., Osborne, L., 2009. Evaluation of *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) as biological control agents of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) on pepper. Biol. Control 49, 91–96.

Bakker, F.M., Sabelis, M.W., 1989. How larvae of *Thrips tabaci* reduce the attack success of phytoseiid predators. Entomol. Exp. Appl. 50, 47–51.

Barry, M., 1994. The costs of crest induction for *Daphnia carinata*. Oecologia 97, 278–288.

Boersma, M., Spaak, P., Du Meester, L., 1998. Predator-mediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. Am. Nat. 152, 237–248.

Bowler, D.E., Yano, S., Amano, H., 2012. The non-consumptive effects of a predator on spider mites depend on predator density. J. Zool. 289, 52–59.

Buitenhuis, R., Shipp, L., Scott-Dupree, C.D., 2010. Intra-guild vs. extra-guild prey: effect on predator fitness and preference of *Amblyseius swirskii* (Athias-Henriot) and *Neoseiulus cucumeris* (Oudemans) (Acari: Phytoseiidae). Bull. Entomol. Res. 100, 167–173.

Choh, Y., Uefune, M., Takabayashi, J., 2010. Predation-related odours reduce oviposition in a herbivorous mite. Exp. Appl. Acarol. 50, 1–8.

Costamagna, A.C., McCornack, B.P., Ragsdale, D.W., 2013. Within-plant bottom-up effects mediate non-consumptive impacts of top-down control of control of soybean aphids. PLoS ONE 8, e56394. http://dx.doi.org/10.1371/journal.pone.0056394.

Ebssa, L., Borgemeister, C., Poehling, H.-M., 2006. Simultaneous application of entomopathogenic nematodes and predatory mites to control western flower thrips *Frankliniella occidentalis*. Biol. Control 39, 66–74.

Fernandez-Ferrari, M.C.F., Schausberger, P., 2013. From repulsion to attraction: species- and spatial context-dependent threat sensitive response of the spider mite *Tetranychus urticae* to predatory mite cues. Naturwissenschaften 100, 541–549.

Fery, R.L., Schalk, J.M., 1991. Resistance in pepper (*Capsicum annum* L.) to western flower thrips [*Frankliniella occidentalis* (Pergande)]. HortScience 26, 1073–1074.

Fischer, S., Pereyra, D., Fernández, L., 2012. Predation ability and non-consumptive effects of *Notonecta sellata* (Heteroptera: Notonectidae) on immature stages of *Culex pipiens* (Diptera: Culicidae). J. Vector Ecol. 37, 245–251.

Gillespie, D.R., 1999. Biological control of thrips (Thysanoptera: Thripidae) on greenhouse cucumber by *Amblyseius cucumeris*. Entomophaga 34, 185–192.

Grostal, P., Dicke, M., 1999. Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. Behav. Ecol. 10, 422–427.

Jacobson, R.J., Croft, P., Fenlon, J., 2001. Suppressing establishment of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) in cucumber crops by prophylactic release of *Amblyseius cucumeris* Oudemans (Acarina: Phytoseiidae). Biocontrol Sci. Tech. 11, 27–34.

Jandricic, S.E., Wraight, S.P., Bennett, K.C., Sanderson, J.P., 2010. Developmental times and life table statistics for the aphid *Aulacorthum solani* (Hemiptera: Aphididae) at six constant temperatures, with recommendations on the application of temperature-dependent development models. Environ. Entomol. 39, 1631–1642.

Lee, D.-H., Nyrop, J.P., Sanderson, J.P., 2011. Avoidance of natural enemies by adult whiteflies, *Bemisia argentifolii*, and effects on host plant choice. Biol. Control 58, 302–309.

Lee, D.-H., Nyrop, J.P., Sanderson, J.P., 2014. Non-consumptive effects of the predatory beetle *Delphastus catalinae* (Coleoptera: Coccinellidae) on habitat use patterns by adult whitefly *Bemisia argentifolii* (Hemiptera: Aleyrodidae). Appl. Entomol. Zool. 49, 599–606.

Lemos, F., Sarmento, R.A., Pallini, A., Dias, C.R., Sabelis, M.W., Janssen, A., 2010. Spider mite web mediates anti-predator behavior. Exp. Appl. Acarol. http://dx.doi.org/10.1007/s1049301093441.

McGregor, R.R., Gillespie, D.R., Quiring, D.M.J., Foisy, M.R.J., 1999. Potential use of *Dicyphus hesperus* Knight (Heteroptera: Miridae) for biological control of pests of greenhouse tomatoes. Biol. Control 18, 104–110.

Mendel, D., Schausberger, P., 2011. Diet-dependent intraguild predation between the predatory mites *Neoseiulus californicus* and *Neoseiulus cucumeris*. J. Appl. Entomol. 135, 311–319.

Morrison, L., 1999. Indirect effects of phorid fly parasitoids on the mechanisms of interspecific competition among ants. Oecologia 121, 113–122.
Nelson, E.H., Matthews, C.E., Rosenheim, J.A., 2004. Predators reduce prey population growth by inducing changes in prey behavior. Ecology 85, 1853–1858.
Peckarsky, B.L., McIntosh, A.R., Taylor, B.W., Dahl, J., 2002. Predator chemicals induce changes in mayfly life history traits: a whole-stream manipulation. Ecology 83, 612–618.
Pozzebon, A., Boaria, A., Duso, C., 2014. Single and combined releases of biological control agents against canopy- and soil-dwelling stages of Frankliniella occidentalis in cyclamen. Biol. Control 12. http://dx.doi.org/10.1007/s10526-014-9641-4.
Preisser, E.L., Bolnick, D.I., Benard, M.F., 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology 86, 501–509.
Ramirez, R.A., Synder, W.E., 2009. Scared sick? Predator-pathogen facilitation enhances exploitation or a shared resource. Ecology 90, 2832–2839.
Relyea, R.A., 2003. Predators come and predators go: the reversibility of predator-induced traits. Ecology 84, 1840–1848.
Rypstra, A.L., Buddle, C.M., 2013. Spider silk reduces insect herbivory. Biol. Lett. 9, 20120948.
SAS Institute Inc, 2011. SAS Software Release 9.3. SAS Institute Inc., Cary, NC.
Schmitz, O.J., Bekerman, A.P., O'Brien, K.M., 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. Ecology 78, 1388–1399.
Schmitz, O.J., Krivan, V., Ovadia, O., 2004. Trophic cascades: the primacy of trait-mediated indirect interactions. Ecol. Lett. 7, 153–163.
Shipp, J.L., Wang, K., 2003. Evaluation of *Amblyseius cucumeris* (Acari: Phytoseiidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) for control of *Frankliniella occidentalis* (Thysanoptera: Thripidae) on greenhouse tomatoes. Biol. Control 28, 271–281.
Shipp, J.L., Whitfield, G.H., 1991. Functional response of the predatory mite *Amblyseius cucumeris* (Acari: Phytoseiidae), on western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). Environ. Entomol. 20, 694–699.
Skaloudova, B., Zemek, R., Krivan, V., 2007. The effect of predation risk on an acarine system. Anim. Behav. 74, 813–821.
Teulon, D.A., Penman, D.R., Ramakers, P.M., 1993. Volatile chemicals for thrips (Thysanoptera: Thripidae) host-finding and applications for thrips pest management. J. Econ. Entomol. 86, 1405–1415.
Thaler, J.S., Griffin, C.A., 2008. Relative importance of consumptive and non-consumptive effects of predators on prey and plant damage: the influence of herbivore ontogeny. Entomol. Exp. Appl. 128, 34–40.
Van der Hoeven, W.A.D., van Rijn, P.C.J., 1990. Factors affecting the attack success of predatory mites on thrips larva. Proc. Sect. Exp. Appl. Entomol. Neth. Entomol. Soc. (NEV) 1, 25–30.
van Maanen, R., Vila, E., Sabelis, M.W., Janssen, A., 2010. Biological control of broad mites (*Polyphagotarsonemus latus*) with the generalist predator *Amblyseius swirskii*. Exp. Appl. Acarol. 52, 29–34.
Van Rijn, P.C.J., 1995. Comparative life history studies of *Frankliniella occidentalis* and *Thrips tabaci* (Thysanoptera: Thripidae) on cucumber. Bull. Entomol. Res. 85, 285–297.
van Tol, R.W.H.M., de Bruin, A., Butler, R.C., Davidson, M.M., Teulon, D.A.J., de Kogel, W.J., 2012. Methyl isonicotinate induces increased walking and take-off behaviour in western flower thrips, Frankliniella occidentalis. Entomol. Exp. Appl. 142, 181–190.
Walzer, A., Schausberger, P., 2009. Non-consumptive effects of predatory mites on thrips and its host plant. Oikos 118, 934–940.
Walzer, A., Schausberger, P., 2011. Threat-sensitive anti-intraguild predation behavior: maternal strategies to reduce offspring predation risk in mites. Anim. Behav. 81, 177–184.
Wardlow, L.R., 1989. Summary of discussions, pp. 1–6. In: van Lenteren, J.C., Wardlow, L.R. (eds.), Proc. IOBC/WPGR Workshop on Biological Control of Pests in Ornamentals in Greenhouses. Aalsmeer, Netherlands, 14–17 Dec 1987. West Palearctic Reg. Section Bull. 1989/XII/3.
Werner, E.E., Peacor, S.D., 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84, 1083–1100.
Wong, S.K., Frank, S.D., 2012. Influence of banker plants and spiders on biological control by *Orius insidiosus* (Heteroptera: Anthocoridae). Biol. Control 63, 181–187.