



## Phytoseiid mites prey effectively on thrips eggs: Evidence from predation trials and molecular analyses



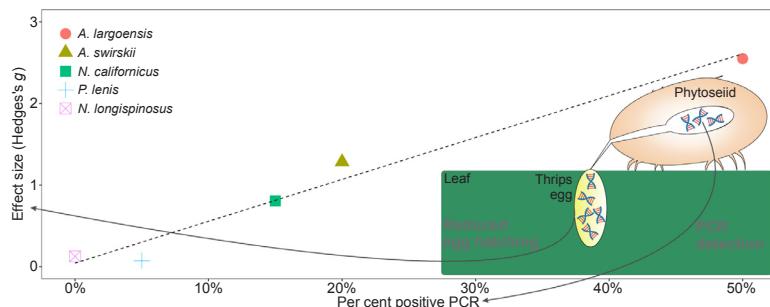
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### GRAPHICAL ABSTRACT



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### ABSTRACT

It is generally assumed that predation by phytoseiid mites (Acarina: Phytoseiidae) on thrips is mainly limited to the first and, to a lesser extent, the second thrips instars. Thrips eggs are imbedded in plant tissue and are supposed to be out of reach of most predators. Using a dual approach combining feeding trials with molecular gut content analysis, we confirmed that eggs of the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), can be vulnerable to attack by a subset of phytoseiid species. Of the five species investigated, the generalist predators *Amblyseius largoensis* and *Amblyseius swirskii* were shown to most readily feed on thrips eggs, followed by *Neoseiulus californicus*, which is classified as a selective predator of tetranychid mites. *Neoseiulus longispinosus* and *Proprioseiopsis lenis* did not or very rarely consume thrips eggs. As substantial reductions of hatching (up to 30%) were observed, predation on eggs should not be ignored when assessing the efficacy of phytoseiids as biological control agents against thrips.

### 1. Introduction

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a cosmopolitan pest and a major problem

in ornamental and vegetable crops in greenhouses as well as in open field crops in warmer areas. Damage to plants is inflicted both directly by feeding activities and indirectly by virus transmission and facilitation of pathogen entry (Jensen, 2000; Lewis, 1997).

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Chemical control of *F. occidentalis* is complicated by its potential to rapidly develop resistance to pesticides, a process which is favored by its short generation time, high fecundity and haplodiploid reproduction system (Jensen, 2000; Reitz, 2009). A safer, healthier and more sustainable management of thrips outbreaks may be attained by the augmentative release or conservation of natural enemies such as phytoseiid predators (van Lenteren et al., 2018). Whereas in greenhouses of temperate areas thrips are increasingly controlled using these biological control agents (van Lenteren et al., 2018), in warmer areas the control of thrips still mainly relies on pesticides. Four phytoseiid species used in this study were collected as part of a project to map the phytoseiid biodiversity in northern Vietnam. As a country facing agricultural intensification, Vietnam requires alternatives for pesticides (Van Hoi et al., 2013) and in search for suitable biological control organisms, native species may be preferred over exotic ones given the potential environmental risks associated with the latter (De Clercq et al., 2011). In addition, native species may be better adapted to local conditions than commercially available species imported for use in augmentative releases. Investigating the potential of native phytoseiids for the biological control of thrips pests in Vietnam was one of the main objectives of this collaborative project.

Both chemical and biological control are hampered by the partly hidden lifestyle of thrips. Larvae and adults show thigmotactic behavior, a preference for residing in tightly enclosed and concealed plant spaces (Reitz, 2009). Female thrips possess a saw-like ovipositor, used to deposit eggs into plant leaves, petioles, bracts, petals and developing fruits (Reitz, 2009). Larvae hatching from these eggs develop through two actively feeding stages, and two quiescent “pupal” stages, in which they prepare for the final stage, the winged adult (Reitz, 2009; Steenbergen et al., 2018). Pupae are non-feeding, soil dwelling stages, although significant numbers can remain on host plants, for example at high relative humidity, or if hosts have a complex floral architecture (Broadbent et al., 2003; Buitenhuis and Shipp, 2008; Reitz, 2009; Steiner et al., 2011).

Because of their potential as biological control agents of small arthropod pests, members of the Phytoseiidae family (Acarina: Mesostigmata) have received increasing attention (Demite et al., 2014; Gerson et al., 2003; McMurtry et al., 2013). Phytoseiids have been classified according to their lifestyles and feeding habits. One of the categories in which candidates for thrips control can be found is the Type III category, a diverse group of species that feed and reproduce on a wide range of prey (McMurtry and Croft, 1997). A subsequent grouping in subtypes is based on the phytoseiids' preferred micro-habitat (McMurtry et al., 2013). *Amblyseius swirskii* Athias-Henriot, for example, belongs to Subtype III-b, containing generalist predators living on glabrous leaves. This species is one of the most successful biocontrol agents in protected cultivation due to its ability to control thrips as well as whiteflies (Calvo et al., 2015). The fact that *A. swirskii* can be mass produced on astigmatid mites at a relatively low cost, while provision of pollen in the field may enhance its efficacy, are keys to its success (Goleva and Zebitz, 2013; Leman and Messelink, 2015; Pijnacker et al., 2016). Also generalist predators from soil or litter habitats (i.e. Subtype III-e) have been used commercially for thrips control, including species such as *Neoseiulus barkeri* (Hughes) and *Neoseiulus cucumeris* (Oudemans) (Gerson et al., 2003; McMurtry et al., 2013). Despite their common occurrence in the soil or litter, both *N. barkeri* and *N. cucumeris* are also found on plants. They have been commercialized for the control of different pest species, including thrips. In addition, effective candidates for thrips control can also be found within the Type IV category of pollen feeding generalists (McMurtry et al., 2013; Messelink et al., 2006).

Depending on the species, phytoseiids rely predominantly on the first larval instar of thrips. Second-instar larvae and pupae are also preyed upon, albeit to a lesser extent (Bakker and Sabelis, 1989; Ghasemzadeh et al., 2017). Predation of thrips eggs by phytoseiids is considered unimportant and has thus been largely ignored in biocontrol

research. However, using an indirect approach, Vangansbeke et al. (2018) hypothesized that certain phytoseiids do feed on thrips eggs: in their laboratory study less thrips hatched from a leaf on which predatory mites had been foraging. Whereas this may be caused by direct predation on the thrips eggs, other mechanisms may also be at play. For instance, phytoseiid feeding on leaf tissue may activate plant defenses, in turn reducing the number of hatching thrips eggs. The objective of the present study was to provide evidence of predation on thrips eggs by selected phytoseiid species, adopting a PCR-based approach to complement observations of predation.

## 2. Materials and methods

### 2.1. Mite collection

Adult female phytoseiids from spider mite- or thrips-infested leaves collected at different locations in the Red River Delta of northern Vietnam were individually transferred to detached *Phaseolus vulgaris* L. leaves in the laboratories of the Vietnam National University of Agriculture, Hanoi, Vietnam. They were supplied with *Tetranychus urticae* Koch, *F. occidentalis* and/or *Typha* spp. pollen (Nutrimite™, Biobest NV, Belgium) as food sources. Part of their progeny was stored in 70% ethanol at  $-20^{\circ}\text{C}$  and used both for molecular characterization or morphological species identification. The remainder of the progeny was kept to establish the respective laboratory colonies for use in the study.

### 2.2. Morphological species identification

The mites were mounted in Hoyer's medium on microscope slides. These were oven-dried and then examined under phase (Leica, DMLB) and interference (Nikon, Eclipse 80i) contrast microscopes. Identification was done by examination of taxonomically relevant structures, and comparison with descriptions and re-descriptions in the literature.

Four phytoseiid species were identified and included in the study. Besides, the commercially available *A. swirskii* was included in the experiments for comparison. Individuals of the latter species were supplied by Biobest NV.

### 2.3. DNA extraction

The DNA extraction procedure was modified from Stavrinides et al. (2010). In short, single mites preserved in ethanol (70%) were transferred to Whatman filter paper until absorbed ethanol had evaporated. Next, mites were transferred to 1.5 ml Eppendorf tubes and incubated at  $56^{\circ}\text{C}$  for 1 min with opened lid. Using an Eppendorf micropesle, the phytoseiids were homogenized in 20  $\mu\text{l}$  of STE-buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) supplemented with 2  $\mu\text{l}$  of proteinase K (10 mg/ml). Next, the homogenate was incubated at  $60^{\circ}\text{C}$  for 30 min, after which proteinase K activity was stopped by heating at  $95^{\circ}\text{C}$  for 5 min. The homogenate was stored at  $-20^{\circ}\text{C}$  until used as DNA-template for PCR-reactions.

### 2.4. Phytoseiid barcoding

The cytochrome *c* oxidase subunit I gene (COI; mtDNA) was used as DNA marker. Phytoseiid-optimized degenerate COI primers were designed, targeting the binding sites of the LCO1490 and HCO2198 primers developed by Folmer et al. (1994). In short, these “universal” invertebrate primers were aligned with the mitochondrial DNA sequences of *Euseius nicholsi* (Ehara & Lee) (GenBank accession number: KM999989.1), *Phytoseiulus persimilis* (Athias-Henriot) (GQ222414.1) and *Galendromus occidentalis* (Nesbitt) (EF221760.1), after which non-identical nucleotides in the LCO1490 & HCO2198 primer sequences were replaced by (degenerate) nucleotides matching the three phytoseiid sequences. These optimized COI primer sequences are shown in

**Table 1**  
Primers used in this study.

Marker	Organism	Primer name	Primer sequence	Approximate amplicon length (bp)	Reference
COI	Phytoseiidae	FoJo_FW	tcwacwaaycayaagayattgg	730	This study, modified from Folmer et al. (1994)
		FoJo_RV	atawacttcdgggtgscaaaaatca		
28S	Phytoseiidae	43F	gctgcgagtgaactggaaatcagcct	1300	(Dowling and O'Connor, 2010; Vicente dos Santos and Tixier, 2017)
		929R	aggtcaccatcttcggc		
COI	<i>F. occidentalis</i>	FOCZ-1F	ttgacttctccaccctt	340	(Zhang et al., 2012)
		FOCZ-1R	tgtatctaaggttcgctg		

**Table 2**  
Thermal conditions for amplification of phytoseiid COI, 28S and *F. occidentalis* COI.

	COI Phytoseiidae	28S Phytoseiidae	COI <i>F. occidentalis</i>
Initial denaturation	95 °C – 2'	95 °C – 2'	95 °C – 2'
10 × Denaturation	95 °C – 30"	95 °C – 60"	/
Annealing	50 °C-1 °C/cycle – 45"	63 °C-1 °C/cycle – 45"	/
35 × Elongation	72 °C – 50"	72 °C – 90"	/
Denaturation	95 °C – 30"	95 °C – 60"	95 °C – 30"
Annealing	40 °C – 45"	53 °C – 45"	55 °C – 45"
Elongation	72 °C – 50"	72 °C – 90"	72 °C – 30"
Final elongation	72 °C – 5'	72 °C – 5'	72 °C – 5'
Hold	16 °C – ∞	16 °C – ∞	16 °C – ∞

**Table 1.** A touchdown PCR approach was chosen to increase PCR sensitivity, specificity and yield (Korbie and Mattick, 2008). The thermal conditions for amplification are represented in Table 2. PCR amplifications were performed using the GoTaq® Flexi DNA Polymerase kit (Promega). Reaction mixtures contained 10 µl 5 × Colorless GoTaq® Flexi Buffer, 3 µl MgCl<sub>2</sub> Solution (25 mM), 1 µl dNTPs (10 µM each), 2.5 µl forward primer (10 µM), 2.5 µl reverse primer (10 µM), 0.25 µl GoTaq® DNA Polymerase (5 µl/µl), 4 µl of mite DNA and water was added to 50 µl. The PCR product was checked using agarose gel electrophoresis. PCR products were purified using the EZNA® Cycle Pure Kit (Omega Bio-Tek), according to the manufacturer's instructions. Bidirectional Sanger sequencing, using the PCR primers, was outsourced to LGC genomics (Germany).

#### 2.5. Thrips egg feeding experiment: assessment of larval hatching

Feeding on thrips eggs by female adults of the five tested phytoseiid species was investigated by determining the number of larvae hatching from *P. vulgaris* leaf discs which had been exposed to foraging phytoseiids for 24 h. To this purpose, leaf discs (4 cm in diameter, n = 40) were placed individually on wet cotton wool, lined with tissue paper inside a closed plastic dish (5 cm in diameter) with wire mesh in the lid to allow air circulation while preventing mites from escaping. Ten two-day-old adult females of *F. occidentalis* were allowed to lay eggs in each leaf disk during 24 h. Six hours after thrips removal, five starved (during 24 h) two-day-old adult predatory mite females were released for 24 h on half of the arenas. The other arenas did not receive phytoseiids and served as controls. The arenas were kept in an incubator throughout the experiment at 25 °C, 16:8 h L:D and 70% humidity. The number of hatched larvae was counted 48 and 72 h after phytoseiid removal.

Numbers of hatched thrips larvae from exposed and unexposed leaf disks were compared using the non-parametric Kruskal-Wallis test, followed by kruskalmc post-hoc tests using the pgirmess package in R studio (Team, 2015).

Effect size was chosen as a metric to represent the difference between the groups exposed and unexposed to phytoseiids, and is expressed as standard deviation units. To this purpose, Cohen's *d* effect

size was determined first by calculating the difference between the mean number of hatched larvae ( $\bar{X}$ ) from arenas unexposed ( $u$ ) versus exposed ( $e$ ) to phytoseiids, divided by the pooled standard deviation (SD) of both groups (Formula 1) (n being sample size) (Borowicz, 2001; Cohen, 1988; Lakens, 2013). Next, Cohen's *d* was corrected (weighted) for the small sample size, resulting in Hedges's *g* (Formula 2) (Cumming, 2013; Lakens, 2013).

$$Cohen's\ d = \frac{\bar{X}_u - \bar{X}_e}{\sqrt{\frac{(n_u - 1)SD_u^2 + (n_e - 1)SD_e^2}{n_u + n_e - 2}}} \quad (1)$$

$$Hedges's\ g = Cohen's\ d \times \left(1 - \frac{3}{4(n_u + n_e) - 9}\right) \quad (2)$$

#### 2.6. DNA detection in gut of *Amblyseius largoensis*: probability curve

To ascertain that reduction of thrips egg survival after the release of phytoseiids was not caused by non-consumptive predator effects, additional evidence was gathered using a molecular gut content analysis approach. To test whether, and for how long, a single thrips egg is detectable in the phytoseiid gut after predation by *Amblyseius largoensis* (Muma), we performed DNA detection probability experiments. This phytoseiid was selected for being one of the predators found in the field sampling, where it was frequently observed on thrips infested plants (see "Section 2.1"). Collecting thrips eggs from plant material is difficult because they are embedded in the plant tissue and difficult to spot under a stereomicroscope. Adult *F. occidentalis* females (two days old) were therefore kept in a cylinder (2 cm in diameter) sealed with stretched Parafilm® on one side and screened with fine wire mesh on the other. *Typha* pollen was added to the cylinder as a food source for the thrips. The cylinder was placed, Parafilm® side down, on an agar plate (agar-agar: 1.3% (w/v) in water). Thrips eggs, laid through the Parafilm® membrane in the agar (Kumm and Moritz, 2010), were collected after 24 h using an insect pin and a fine brush. The eggs were offered to starved (during 24 h) two-day-old adult females of *A. largoensis* on small leaf arenas. The predators were monitored under a binocular microscope and time was recorded as soon as they abandoned a consumed egg. The mites were then transferred to an empty arena and after 0, 30, 60, 90 or 120 min, they were transferred to an Eppendorf® tube containing 90% ethanol and stored at -20 °C until DNA extraction. Ten specimens were analyzed for each time point. Thrips (egg) DNA in the phytoseiid samples was then amplified using *F. occidentalis*-specific primers (FOCZ-1F and FOCZ-1R (Zhang et al., 2012)). Unfed phytoseiids were included as negative controls. The phytoseiid 28S region (Table 1) was amplified as an internal control, while a *F. occidentalis* DNA sample was included as a positive control. Eventually, the "DS<sub>50</sub>" was calculated; this is the time after which half of the predators of the cohort that fed at the same time tested positive for the presence of a species of prey, considering that the rate of prey decay is usually exponential (Gagnon et al., 2011; Greenstone et al., 2007; Pérez-Sayas et al., 2015).

Prey DNA detection probability may vary between phytoseiid species (Pérez-Sayas et al., 2015). Instead of constructing a full probability curve as done for *A. largoensis* specimens (n = 10) from the four other

phytoseiid species were offered single thrips eggs dissected from agar, and thrips DNA detectability success was evaluated after a digestion time of 85 min only (i.e. the DS<sub>50</sub> of *A. largoensis*, see Section 3.3).

### 2.7. Thrips egg feeding experiment: detection of thrips DNA in phytoseiid gut

*P. vulgaris* leaf discs (4 cm in diameter, n = 40) were placed on wet cotton wool, lined with tissue paper, and individually placed inside a closed plastic dish (5 cm in diameter) with wire mesh in the lid. Ten *F. occidentalis* adult females (two days old) were allowed to lay eggs in half of the leaf disks during 24 h. The remaining disks served as controls and received ten two-day-old second-instar thrips larvae instead, assumed to cause similar levels of (DNA) contamination as the adults, yet without laying eggs. Twenty-four hours after removal of thrips adults or larvae, a single starved (during 24 h) phytoseiid was released on each of the arenas. After 2 h of foraging, each phytoseiid individual was transferred to 90% ethanol and stored at -20 °C until used for DNA extraction. This experiment was independently performed for all five phytoseiid species tested. PCR conditions were identical to those in the above experiment (see Section 2.6).

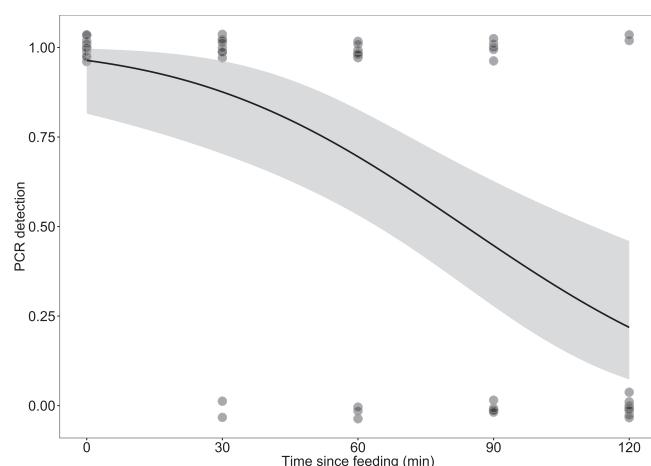
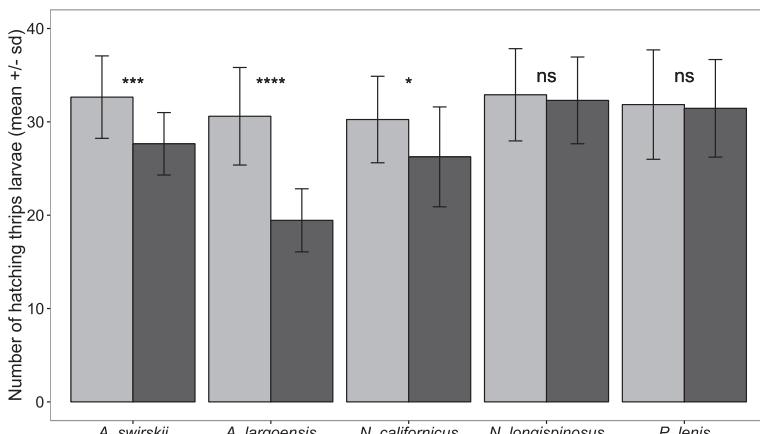
## 3. Results

### 3.1. Species identification and barcoding

Morphological species identification revealed that the following phytoseiids had been collected in Vietnam: *A. largoensis*, *Proprioseiopsis lenis* (Corpuz and Rimando, 1966), *Neoseiulus longispinosus* (Evans, 1952) and *Neoseiulus californicus* (McGregor, 1954). Their corresponding COI sequences have been submitted at GenBank (respective Accession Numbers: MK577645, MK577646, MK577647, MK577648).

### 3.2. Thrips egg feeding experiment: assessment of larval hatching

Substantially fewer thrips hatched from eggs in leaf discs exposed to *A. swirskii*, *A. largoensis* or *N. californicus* compared to control discs not exposed to predatory mites (Fig. 1). Leaf discs with *A. largoensis* had the lowest egg hatch (mean = 19.5) compared to the corresponding controls (mean = 30.6, p < 0.0001). Thrips eggs on leaf discs with *A. swirskii* and *N. californicus* resulted in a mean of 27.7 and 26.3 larvae hatching from eggs compared to control leaf discs with a mean of 32.7 (p < 0.0001) and 30.3 (p < 0.02) eggs that hatched, respectively. Conversely, no difference was observed after *N. longispinosus* (p = 0.92) or *P. lenis* (p = 0.98) foraging. There was no difference in thrips egg hatching from control leaf disks between the five experiments (Kruskal-Wallis test, p = 0.39). It should be noted that phytoseiids could not have preyed upon thrips larvae instead of eggs given that no hatching was observed during the time course while predatory mites were on the



**Fig. 2.** Logistic regression curve of the PCR detectability of DNA from a (dissected) single thrips egg after predation by individual *A. largoensis* specimens as a function of the time passed since feeding. The shaded area corresponds to the 95% confidence interval. Raw data are shown by transparent dots representing samples that tested positive (PCR detection = 1) or negative (PCR detection = 0) for *F. occidentalis* DNA. Random jitter was added to the y coordinates to make overlapping data points visible.

leaf discs.

The effect size (Hedges's *g*) of phytoseiid foraging on thrips egg survival was considered to be large (*g* > 0.8) for *A. swirskii*, *A. largoensis* and *N. californicus*, whereas *N. longispinosus* and *P. lenis* release had no effect (*g* < 0.2). Hedges's *g* was plotted against the PCR detection probability (Fig. 3, see Section 3.4).

### 3.3. DNA detection in gut of *Amblyseius largoensis*: probability curve

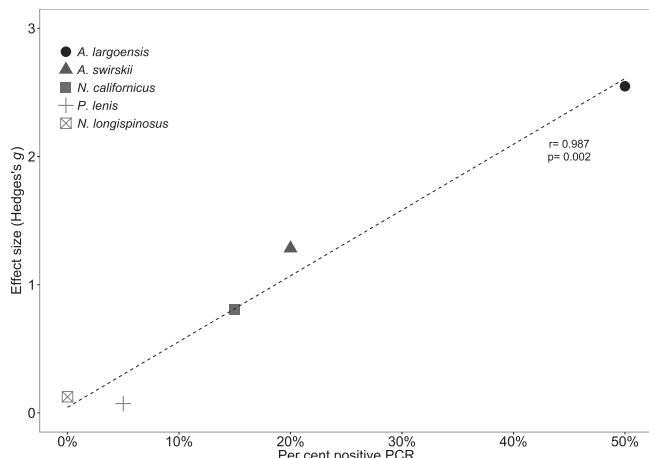
To evaluate if, and for how long, DNA of single *F. occidentalis* eggs is detectable after it has been fed upon by a phytoseiid, we performed feeding trials followed by DNA extraction and PCR analysis. Fig. 2 shows a logistic regression curve of the detection success from the time each *A. largoensis* specimen ended feeding on a (dissected) thrips egg in function of digestion time. The DS<sub>50</sub> of a single thrips egg ingested by *A. largoensis* amounted to 85 min.

When *A. swirskii*, *N. californicus* and *P. lenis* were offered single dissected thrips eggs, thrips DNA could still be detected in 60%, 60% and 80% of the specimens, respectively. *Neoseiulus longispinosus* was also included in the experiment, yet refused the eggs as prey.

### 3.4. Thrips egg feeding experiment: detection of thrips DNA in phytoseiid gut

*Frankliniella occidentalis* DNA from eggs deposited in leaf tissue was

**Fig. 1.** Histogram showing thrips egg survival (mean ± sd) as determined by the number of thrips larvae hatching from leaf arenas after temporary foraging (24 h) by different phytoseiid species (black bar). Leaf arenas on which no phytoseiids were released were used for control counts (grey bar). Asterisks indicate the significance level as determined by Kruskal-Wallis test (ns = not significant; \* p ≤ 0.05, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001, \*\*\*\* p ≤ 0.0001).



**Fig. 3.** Correlation between effect size (Hedges's  $g$ ) and per cent PCR detection of *F. occidentalis* DNA in phytoseiid specimens. Hedges's  $g$  represents the (corrected) standardized difference between means of thrips larvae hatching from leaf arenas with versus without temporary phytoseiid foraging. Per cent positive PCR represents the percentage of individual phytoseiid specimens from which thrips (egg) DNA could be detected after foraging on leaf arenas in which thrips were allowed to oviposit.

detected in *A. swirskii* (20%), *A. largoensis* (50%), *N. californicus* (15%) and *P. lenis* (5%), but not in *N. longispinosus* (0%). Thrips DNA was never detected in phytoseiid specimens from the control treatment, i.e. phytoseiids that were released on leaf disks on which thrips larvae had been foraging beforehand, instead of adult female thrips. No thrips egg hatching was observed during (and 12 h following) the experiment, so phytoseiids could not have preyed upon larvae instead of eggs.

The incidence of thrips DNA detection in phytoseiids (positive PCR) was plotted against the size of the effect (Hedges's  $g$  effect size) of phytoseiid foraging on thrips egg hatching numbers (Fig. 3). We observed a significant correlation between PCR detection and effect size (Pearson's  $r = 0.987$ ,  $p = 0.002$ ).

#### 4. Discussion

Predation of thrips eggs by phytoseiids is generally considered unimportant and has thus been largely ignored in biocontrol research. For example, when modeling predator-prey population dynamics, Magalhães et al. (2007) categorized *F. occidentalis* eggs as non-visible and thus invulnerable to *N. cucumeris* attack. According to Bakker and Sabelis (1989), thrips eggs, like adults, easily escape attack. One of the few papers reporting on thrips egg feeding by phytoseiids is Jacobson et al. (2001), stating that *N. cucumeris* adults only feed on thrips eggs and first instar nymphs. However, no such evidence could be found in the two papers cited by Jacobson et al. (2001) to substantiate this claim (i.e. Bakker and Sabelis, 1989; Gillespie and Ramey, 1988). Indeed, *N. cucumeris* was reported to feed on *Tetranychus* eggs instead of thrips eggs (Gillespie and Ramey, 1988). Encouraged by the feeding trials by Vangansbeke et al. (2018), we aimed to provide proof of phytoseiid predation on *F. occidentalis* eggs imbedded in leaf tissue.

The observation that foraging by *A. swirskii*, *A. largoensis* or *N. californicus* adults significantly reduced the number of *F. occidentalis* eggs hatching from bean leaf disks in our bioassays could lead to the conclusion that these phytoseiids were able to detect and prey upon the eggs hidden in the leaf tissue. However, determining whether the foraging results in a reduction of egg hatchability is an indirect and inconclusive way of showing that phytoseiids feed on thrips eggs. Indeed, non-consumptive predator effects could be at the basis of this reduction instead.

As an alternative, it could be hypothesized that the phenomenon at the basis of a reduced thrips egg survival is the feeding by the

phytoseiids on leaf tissue. Such feeding from plant tissue has indeed been shown for a number of phytoseiids (Magalhaes and Bakker, 2002). Females of *Typhlodromus pyri* Scheuten, for example, were shown to cause scars on apple leaves. The negative relationship between scar number and the availability of alternative food sources such as pollen and prey suggested that these scars are caused by nutrient retrieval from the plant (Sengonca et al., 2004). Similarly, *Typhlodromalus aripo* De Leon feeds upon leaves of cassava (Magalhaes and Bakker, 2002). Also *Euseius scutalis* (Athias-Henriot) was shown to take up cell content from plant leaves, although damage remained limited to single cells, leaving neighboring cells mostly intact (Adar et al., 2015). *Amblyseius swirskii* has been shown by Adar et al. (2012) to be able to pierce plant cells as well, although it was deemed unable to extract liquids from them. Such plant piercing or feeding by predatory mites could then, hypothetically, induce plant defense responses, in turn affecting thrips egg survival. Whether leaf puncturing by phytoseiids alerts the plant defense system has, to our knowledge, not yet been investigated. On the other hand, the predatory bug *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae), which is used for the control of *F. occidentalis* in sweet pepper, oviposits and also feeds on plant tissue, and it has been shown to induce plant defense signaling, including the jasmonic acid and salicylic acid signaling pathway (Bouagga et al., 2018; De Puyseleyr et al., 2011). Further, it has been shown that the number of *F. occidentalis* larvae hatching from defense-induced plants could be significantly lower than from control plants (Agrawal and Klein, 2000; De Puyseleyr et al., 2011).

To exclude potential causes for the reduced thrips egg hatching other than the direct effect of phytoseiid predation, we relied on the PCR-based approach. To begin with, we observed that certain phytoseiids feed on thrips eggs that had been isolated from agar plates, and that thrips DNA can be amplified within a limited time after feeding (e.g. the DS<sub>50</sub> of a single egg in *A. largoensis* is 85 min). With this knowledge in mind, phytoseiids were released on bean leaf arenas into which thrips were allowed to lay eggs. Eggs and, as such, predation of eggs by the phytoseiids, could not be observed under a stereomicroscope, yet, certain phytoseiids tested positive for thrips DNA after a 24 h foraging period. Based on the molecular gut content analysis, of the five species tested, *A. largoensis* most readily fed on *F. occidentalis* eggs, followed by *A. swirskii* and *N. californicus*. The results of this study further suggest that *N. longispinosus* and *P. lenis* do not or only very rarely feed on *F. occidentalis* eggs. McMurtry et al. (2013) did not refer to *A. largoensis*, but most certainly it should be placed with *A. swirskii* and other species of the genus as a generalist predator on plants with glabrous leaves. As to *N. longispinosus*, our findings are in concert with the classification of this species by McMurtry et al. (2013) as a selective predator of tetranychid mites. *Neoseiulus californicus* is also classified by those authors within that same group, however. Despite its apparent more generalist behavior as compared with *N. longispinosus*, the latter authors decided to place it in that group because of the high frequency with which it is found in association with heavily webbing spider mites. Species of *Proprioseiopsis* are commonly found in the soil/litter habitats, although some species are also common on plants. Despite being widespread (De Moraes et al., 2004), little has been reported about the biology of the mites in this group (McMurtry et al., 2013).

Whereas the lifestyle category (McMurtry et al., 2013) may indeed be a predictor of the species-dependent differences in predation on thrips eggs, (a combination of) different specific factors may be at the base of a phytoseiid's ability to successfully feed on thrips eggs. For instance, the ability to feed on eggs imbedded in leaf tissue could be related to the ability to feed on plant tissue, which is in turn determined by cheliceral morphology (Adar et al., 2015). However, whereas *A. swirskii* has been deemed to be unable to feed on plant tissue (Adar et al., 2015), it can feed on thrips eggs. An alternative explanation may be found in the intensity of the searching activity of a phytoseiid. Mites with a high search rate, such as *A. swirskii* (Messelink et al., 2006), may be more likely to encounter the motionless thrips eggs than 'sit and wait

predators'. A relevant note here is that *N. longispinosus*, which did not prey upon "hidden" thrips eggs in our feeding trials (Fig. 3), also refused to consume thrips eggs that were isolated from the leaf tissue. The process of locating and recognizing prey is suggested to rely on chemo- and/or mechanoreceptors, involving several types of setae on the anterior tarsi (Gilstrap and Friese, 1985; Jackson and Ford, 1973). Potentially, *N. longispinosus*, being a selective predator of tetranychid mites (McMurtry et al., 2013), does not respond to thrips-specific stimuli. Clearly, more research is needed in this regard.

Feeding by certain phytoseiids on thrips eggs could be quite substantial. In the thrips egg feeding experiment in which we detected thrips DNA in the phytoseiid gut, for example, 50% of the previously starved *A. largoensis* specimens tested positive for thrips (egg) DNA after only 2 h of foraging. In addition, this species also severely reduced the number of thrips larvae hatching from leaf arenas (Fig. 3 and Fig. 1). Care should be taken, as the interpretation of predator impact may be biased if only detectability is taken into account, and these data should then be weighed by the relative detectability periods (Greenstone et al., 2014). Differential detection half-lives (DS<sub>50</sub>) in distinct phytoseiids have been observed before (Pérez-Sayas et al., 2015), and may be due to various nonexclusive traits, including morpho-physiological features in the digestive system (Akimov and Starovir, 1980) as well as different pre- and post-oral digestive processes (Greenstone et al., 2014; Pérez-Sayas et al., 2015). Interestingly, we observed the detection half-life of single (dissected) thrips eggs to be shortest in the phytoseiids that more readily fed on eggs (deposited in leaves). Digestion speed may indeed be related to the level of specialization of phytoseiids (Akimov and Starovir, 1980; Pérez-Sayas et al., 2015). In light of this, the impact of e.g. *A. largoensis*, as assessed by the per cent positive PCRs, may be even higher as only half of the ingested thrips eggs would still be detectable 85 min after feeding (Fig. 2).

Thrips egg feeding by phytoseiids may have direct and potentially substantial consequences on thrips population numbers in the field and thus add to their biological control potential. Indeed, relatively large differences between the number of thrips eggs hatching from leaves with versus without phytoseiids were observed (Fig. 1). For example, *A. largoensis* foraging reduced the number of hatching eggs by over 30%. Other phytoseiids, such as *Euseius gallicus* Kreiter & Tixier (Vangansbeke et al., 2018), could be even more efficient in this regard and an assessment of additional species is therefore warranted. The consumption of eggs rather than larvae may be particularly valuable as it eliminates new herbivores before they inflict feeding damage. Furthermore, because of the complex thigmotactic life cycle of *F. occidentalis*, it is advisable to target different life stages, using different beneficial agents, in order to achieve successful control (Buitenhuis and Shipp, 2008). The identification of phytoseiids which feed on thrips eggs, instead of or in addition to thrips larvae, could therefore be at the basis of improving the efficacy of thrips management. As thrips eggs protrude more and more from the leaf surface during development (Loomans and Heijboer, 1999), also the influence of leaf type and egg age deserve additional attention during this evaluation process. Furthermore, given that thrips do not exclusively deposit eggs in leaves (Reitz, 2009), and phytoseiids may not be evenly distributed throughout the plant (Messelink et al., 2006), it is advisable to study whole plants rather than specific plant tissues.

Another merit of the ability to feed on thrips eggs is worth highlighting. Phytoseiid mites face difficulties subduing their thrips prey because of the larval defensive behavior. Due to size constraints, predatory mites are mainly confined to attacking first-instar larvae while second instars are rarely preyed upon (Bakker and Sabelis, 1989; Magalhães et al., 2007; Van der Hoeven and Van Rijn, 1990; Wimmer et al., 2008). The difficulties in catching a larger and defensive prey may be even more pronounced for juvenile phytoseiids, potentially causing high juvenile mortality (Vantornhout et al., 2004; Wimmer et al., 2008). In the absence of alternative food sources such as pollen, the ability to feed on (largely) hidden, yet small and defenseless thrips

eggs, may enhance the survival of phytoseiids. We expect this to be the case for juvenile predators in particular. The indirect negative effect on thrips populations by egg predation, i.e. via an increase in phytoseiid juvenile survival, may be significant. Additional research is required to substantiate latter hypothesis, but it is advisable to include the different stages of both pest and predator in predation trials.

In conclusion, our molecular gut content analyses confirm that adults of certain phytoseiids (i.e. *A. largoensis*, *A. swirskii* and *N. californicus*) effectively feed on *F. occidentalis* eggs imbedded in leaf tissue. The ability to do so might be a valuable asset, increasing their efficacy as biological control agents.

## CRediT authorship contribution statement

**Viet Ha Nguyen:** Conceptualization, Methodology, Investigation. **Wim Jonckheere:** Conceptualization, Methodology, Investigation, Writing - original draft. **Duc Tung Nguyen:** Supervision, Funding acquisition, Project Administration. **Gilberto José de Moraes:** Investigation, Writing - review & editing. **Thomas Van Leeuwen:** Supervision, Funding acquisition. **Patrick De Clercq:** Supervision, Funding acquisition, Project Administration, Writing - review & editing.

## Declaration of Competing Interest

None.

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