Predation rate of *Thrips tabaci* larvae by *Neoseiulus cucumeris* is influenced by prey density and presence of a host plant

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Abstract Prey consumption rate by natural enemies may be affected by the prey's host plant and prey density. The predation rate of the mite *Neoseiulus cucumeris* at different densities of first-instar *Thrips tabaci* larvae in the presence (onion) or absence (green plastic) of a host was measured. In the first experiment using a disc bioassay, *N. cucumeris* exhibited a type-II density-dependent functional response to prey on both disc types. Prey consumption was reduced on onion relative to plastic. A second experiment using onion bulbs and green plastic bulbs indicated a decrease in the consumption rate on plastic bulbs compared with plastic discs. The survival or recapture of *N. cucumeris* on onion bulbs was less than 7% and no prey consumption data were obtained. The results indicate that onion as a host plant has a negative effect on consumption of *T. tabaci* by *N. cucumeris* and on survival of the predator mite.

Keywords predatory mite, onion, biological control, onion thrips, prey, tritrophic interactions.

INTRODUCTION

Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), is a cosmopolitan species (Lewis 1997), feeding on a wide variety of vegetable plants, small grains, field crops and weeds (Summerville 1933). It is an important pest on onions (*Allium cepa* L.) in New Zealand (Martin et al. 2003; Workman et al. 2007) and worldwide (Diaz-Montano et al. 2011). Despite chemical control programmes undertaken during the growing season, harvested onion bulbs in storage are regularly infested by onion thrips and damage from thrips feeding can cause the onions to be downgraded in quality and market value (Mayer et al. 1987; Workman & Martin 2002), making it an important issue for New Zealand onion producers (Wood 2001). In-storage control options would potentially produce higher quality onion bulbs.

The predatory mite *Neoseiulus cucumeris* (Oudemans) (Acarina: Phytoseiidae) is considered a polyphagous predator, feeding on a number of mites and insect species (McMurtry & Croft 1997). World-wide it is used with some success for biocontrol of thrips such as onion thrips and *Frankliniella occidentalis* (Pergande) on vegetables and ornamentals in greenhouses (Ramakers 1988; Gillespie 1989; Brødsgaard & Hansen 1992; Jacobson et al. 2001; Williams 2001). Additionally, preliminary research undertaken in Australia suggests that *N. cucumeris* can play a significant role in controlling onion thrips in stored onion bulbs when adding the mites directly into the onion crates in the storage facility (Baker 2007; Baker & Powis 2009). The use (either introduced

or naturally present) of predatory mites in storage facilities against serious pest insects has previously shown promise for several predators, such as *Cheyletus eruditus* (Acari: Cheyletidae) against *Acarus siro* and *Glycyphagus destructor* in stored grain in Canada (Pulpán & Verner 1965), *Pyemotes tritici* (Acari: Pyemotidae) for the management of the groundnut beetle *Caryedon serratus*, a major pest of peanuts in the Congo (Matokot et al. 1987) and more recently *Tyrophagus putrescentiae* (Acari: Acaridae) for management of *Lasioderma serricorne* in stored tobacco in Brazil (Canevari et al. 2012). The evidence that *N. cucumeris* could reduce onion thrips in stored onions was based on spreading mites from commercially available sachets over bins of stored onions and determining the number of thrips/bulb and percentage of different graded onions in treated and untreated bins (Baker 2007; Baker & Powis 2009). The efficacy of predation by the mite was not directly evaluated and prey consumption rate by natural enemies may be affected by the prey's host plant and prey density. The purpose of this study was to evaluate *N. cucumeris* predation of first instar onion thrips larvae in the presence or absence of onion host plant material.

MATERIALS AND METHODS

Insects

Onion thrips were reared on leek (*Allium porrum* L.) in the entomology lab at Plant & Food Research Limited (PFR), Lincoln, at room temperature (19–22°C) and under natural sunlight. The colony was established from individuals obtained from a colony maintained at the PFR campus at Mt Albert, Auckland (for details see Martin & Workman 2006). Glass preserving jars (0.5 litre) served as rearing containers. In the jar lid, a 2–4 cm diameter opening was cut and covered with thrips-proof mesh (80 µm) for ventilation. The bottoms of the jars were lined with a 0.5–1 cm deep layer of cut hand-towel paper pieces to provide a pupation site for the thrips. Clean segments of leek stalks (5 × 2 cm) were added to the jars twice weekly as food and for oviposition sites. First instar larvae of the onion thrips are the most vulnerable stage

to predators (Bakker & Sabelis 1989) and was the stage used as prey in the experiments. The larvae were discriminated by visual means; first instar larvae are white or hyaline and 0.35–0.38 mm long (Lewis 1973).

Neoseiulus cucumeris mites (mixed life stages) were obtained from Zonda Beneficials Ltd, Pukekohe. The mites were delivered in a bran medium with *Tyrophagus* spp. mites as the alternative prey source. At PFR the mites were stored in the bran medium at 16 ± 1°C and 16:8 h light:dark cycle unless otherwise specified. Prior to any experiments the required number of mites was removed from the bran medium and placed with first instar onion thrips larvae as prey on plastic discs (5 cm in diameter) placed on water-saturated filter paper in a Petri dish for 3 days to pre-condition the mites.

Disc arena assay

The predation arenas consisted of discs (2.5 cm in diameter) placed on water-saturated filter paper (4 cm in diameter) in a Petri dish (5 cm in diameter) with a lid. The saturated filter paper maintained a high relative humidity, provided available water and prevented both thrips and mites from walking off the disc arena. Discs were made from (1) green plastic (non-host) and (2) the second fleshy scale of brown onions (host plant material). The green plastic discs were made from sheets of flexible plastic, approximately 0.3 mm in thickness, with a smooth matt surface that did not absorb any moisture from the water-saturated filter paper. The onions were purchased from a local supermarket (cultivar not known). First instar onion thrips larvae were transferred to the discs by a fine camel-hair brush directly from the laboratory colony. Prey densities of 1, 3, 5, 8 or 16 larvae/disc were tested. A single *N. cucumeris* predatory mite that had been starved for 24 h (access to water only) was added to each disc. The discs were left for 24 h at 22 ± 1°C, 60% RH and 16:8 h light:dark cycle. After 24 h, the predation arenas were examined under a stereomicroscope (>40× magnification) to record the number of fed-upon thrips larvae. Any discs with missing or dead mites, or where escape of the mites and/or thrips larvae might have occurred

through water loss resulting in the filter paper drying out, or where the disc was in contact with the inner wall of Petri dish, were not scored or included in the analyses. The experiment was repeated over 6 days (6 replicates), each day with a new mite, and the position of the treatments was randomised.

Whole bulb assay

Small brown onion bulbs (pickling onions, cultivar not known, maximum 4.5 cm high and 3.5 cm wide) from a local supermarket were placed individually in plastic containers (4×4×5 cm) with tight fitting lids. The bulbs' outer most layer (dry brown skin) varied greatly, so this layer was removed to achieve a uniform outer surface between the treatments and replicates. The neck of the onion was also cut in a straight angle to a height of 1–2 mm above the bulb for all replicates to minimise variability between the individual onions and to create an opening for both thrips and mites to enter the onion's inner layers. Artificial bulbs (non-host) were created using green plastic sheets. Circular pieces (10 cm in diameter) were folded around a small cotton ball (1–1.5 cm in diameter) and secured with a rubber band above the cotton ball. This mimicked the bulb shape of the onion bulbs and provided folds within which the larvae could hide. The artificial bulbs were placed within plastic containers (2×2×7 cm) with tight fitting lids and with 0.2 ml of water in the bottom to ensure high humidity within the container. This was not necessary for the onion bulbs as water droplets always formed on the inside of the container with onion bulbs. Based on consumption achieved in the disc arena assay by a single mite, prey densities (first instar onion thrips larvae) of 1, 3, 5, 7 and 10 larvae/bulb were tested. The thrips larvae were transferred by a fine camel-hair brush on to an onion bulb or an artificial bulb and left for 6 h at room temperature to allow the thrips time to enter the bulbs. All mites used were pre-conditioned on onion thrips larvae as described above prior to the experiment. After 6 h a single predatory mite that had been starved (water only) for 24 h prior was then introduced onto each bulb. The bulbs were left for 24 h at $22 \pm 1^\circ\text{C}$ and 16:8 h

light:dark cycle. After 24 h, the bulbs (outer surface and inside layers) were examined under a stereo-microscope ($>40\times$ magnification) to record the number of fed-upon thrips larvae. Any bulbs with missing or dead mites were not scored or included in the analyses. The experiment was repeated over 6 days (6 replicates) and the position of the treatments was randomised.

Statistical analysis

The percentage of onion thrips larvae consumed (numbers consumed out of numbers added) by *N. cucumeris* alive after 24 h were analysed with a binomial generalized linear model with a logit link (GLM, McCullagh & Nelder 1989). Formal analysis was carried out only of data from the plastic discs, onion discs and plastic bulbs, because of the lack of data from the onion bulbs (i.e. only 7% *N. cucumeris* alive after 24 h). This analysis included comparisons between the disc types, number of added larvae, and the interaction between these. These effects were assessed with F-tests within an analysis of deviance done as part of the analysis. The percentage of larvae consumed and associated 95% confidence limits were obtained on the transformed (logit) scale, and back-transformed. These were then converted into mean numbers consumed by multiplying by the initial numbers added to the discs. To confirm the type of functional response (Type II or III) (Trexler & Travis 1993), the data were examined further with logistic regression analyses: this is exactly the same analysis as the before mentioned binomial GLM, except that the numbers added are included not as a five level factor, but as a linear function of numbers added. In the present case, there were five different levels of thrips added, so a Quartic function was used. This five-parameter model exactly models the pattern in the mean percentage consumed from the initial analysis:

$$\frac{N_e}{N} = \frac{\exp(P_0 + P_1 N + P_2 N^2 + P_3 N^3 + P_4 N^4)}{1 + (P_0 + P_1 N + P_2 N^2 + P_3 N^3 + P_4 N^4)}$$

where N_e is the number of first instar onion thrips larvae consumed, N is the initial number of larvae added to the discs and P_0 , P_1 , P_2 , P_3 and

P_4 are the parameters to be estimated. If the linear parameter P_1 is negative, a type II functional response is indicated, whereas a positive linear parameter indicates a type III functional response (Juliano 2001).

All analyses were carried out with GenStat v. 16.

RESULTS

Disc arena assay

Consumption of first instar onion thrips larvae by *N. cucumeris* was substantially reduced on onion (host plant material) relative to plastic (non-host) ($P=0.008$). Data on the number of thrips consumed by treatment are provided in Figure 1. On average, 47% of onion thrips larvae were consumed from plastic discs compared with an average of only 24% from onion discs. This effect was consistent for all initial numbers of onion thrips larvae ($P=0.588$ for the disc type by numbers added interaction). The most first instar onion thrips larvae consumed over the 24-h period was 9 on a plastic disc and 6 on an onion disc (16 thrips larvae added in total on both discs). The percentage of onion thrips larvae consumed decreased with the number initially added ($P=0.006$), decreasing from 84% with 1 added thrips to an average 25% with 16 added thrips, suggesting a type II functional model. Upon fitting this model, it was found that the Quadratic, Cubic and Quartic parameters (P_2, P_3, P_4) were not significant ($0.1 < P < 0.9$ for P_2, P_3, P_4). Thus the model was re-fitted with just the constant (P_0) and linear parameter (P_1). The results confirmed a type II functional response for *N. cucumeris* to first instar onion thrips larvae on both the plastic and the onion discs (Table 1).

Whole bulb assay

Of the 30 *N. cucumeris* mites added onto the whole onion bulbs, only 2 were found alive at assessment after 24 h. The majority (75%) of the 28 dead/missing mites were found on the outer layer of the onion bulb. When it was established that the mite was dead, no further search for the added first instar thrips larvae was undertaken. Data from the surviving mites were insufficient to allow an assessment of any relationship between initial larval numbers and survival of larvae on

the onion bulbs or for a comparison between bulb types. However, a number of the larvae introduced to the whole onion bulb were found dead both on the outer layer and within the onion bulb layers, indicating that onion bulb is a poor host for both the mite and the onion thrips larvae. For plastic bulbs, 20 of the 30 mites were found alive after 24 h. The percentage of first instar onion thrips larvae consumed by the mites on the artificial plastic bulb did not vary significantly with initial numbers of larvae ($P=0.533$), ranging from 25% to 60% consumed (Table 2).

Table 1 Parameters from logistic regression analyses of the proportion of first instar onion thrips (*Thrips tabaci*) larvae consumed by one adult *Neoseiulus cucumeris* on either green plastic discs (non-host) or onion discs (host plant material).

Disc type	Parameter	Estimate	SE
Green plastic	Constant (P_0)	1.494	0.535
	Linear (P_1)	-0.147	0.044
Onion	Constant (P_0)	-0.254	0.546
	Linear (P_1)	-0.084	0.048

Figure 1 Mean numbers of first instar onion thrips (*Thrips tabaci*) larvae consumed by one adult *Neoseiulus cucumeris* on either green plastic discs (non-host) or onion discs (host plant material) over a 24 h period at $22 \pm 1^\circ\text{C}$. Error bars are 95% confidence limits.

Table 2 Mean numbers (95% confidence limits) of first instar onion thrips (*Thrips tabaci*) larvae consumed by one adult *Neoseiulus cucumeris* on green plastic bulbs (non-host) over a 24 h period at $22 \pm 1^\circ\text{C}$.

Larvae added	Number consumed
1	0.3 (0.0,0.9)
3	1.5 (0.5,2.5)
5	3.0 (1.8,4.0)
7	4.0 (1.7,5.9)
10	3.8 (2.0,5.8)

Consumption rate on disc arena versus whole bulb

The quantity of larvae consumed over 24 h on the plastic bulb was noticeably lower than on the discs. When only one first-instar onion thrips larva was added, the percentage consumed was 100% on the plastic discs but only 25% on the plastic bulb. This difference decreased with more larvae added to each bulb.

DISCUSSION

The results suggest that onion bulbs, at least when damaged, may have a detrimental effect on survival of *N. cucumeris* and on the consumption of onion thrips larvae by *N. cucumeris*, affecting the functional response itself, but not the type of functional response. That the host plant does not influence the type of functional response of *N. cucumeris* has previously been found for onion thrips on leaf discs of cucumber, egg plant and capsicum (Madadi et al. 2007). Similarly, a lack of host plant influence on the functional response of *N. cucumeris* was also reported when the mite preyed on western flower thrips (*F. occidentalis*) on cucumber and capsicum leaf discs (Shipp & Whitfield 1991). A type II functional response indicates an inverse density-dependent predation (Juliano 2001). Most invertebrate predators and most parasitoids exhibit the type II functional response (Price et al. 2011). However, uncontrolled and highly variable field conditions could radically change the functional response of predators (Farhadi et

al. 2010), and functional response studies in small laboratory arenas should be interpreted with care in regards to representing field conditions. In regards to onions in storage bins, prey patchiness and environmental complexities (abiotic and biotic factors) may influence the prey efficiency of *N. cucumeris* on thrips larvae.

The mean consumption of onion thrips larvae by *N. cucumeris* was overall (both for plastic discs and onion discs) lower than that previously reported by Madadi et al. (2007) on cucumber, eggplant and capsicum leaf discs. This is despite the arena size in the present disc experiment being smaller (2.5 cm in diameter compared with 4 cm in diameter) and the mites being pre-conditioned on onion thrips larvae prior to the experiment. In addition to using different host plant material, Madadi et al. (2007) only used mated female adult mites whereas the present study did not discriminate between mated and unmated or male and female adult mites. Shipp & Whitfield (1991) found that mated female adults were the most effective predatory stage, whereas all male stages were ineffective predators. This could partially account for the overall low consumption of onion thrips larvae on both plastic and onion discs. Additionally, the effectiveness of *N. cucumeris* against onion thrips larvae may be influenced by the chemical properties of the onion (Vet & Dicke 1992).

The mites consumed fewer thrips larvae on the plastic bulb than on the plastic disc when the prey density was low (<5 larvae, Figure 1 & Table 2). This result was not surprising given that the mites had a larger area to search and the bulbs provided a more extensive and textured environment for the larvae to hide in. As prey density increased the difference diminished, likely because the mite would more readily encounter the thrips larvae and consumption would be more limited by feeding time on the individual larvae. However, the maximum mean number of larvae consumed on the plastic bulbs was four and the number did not increase with increased density, suggesting that a higher searching time for *N. cucumeris* on the bulbs limited the consumption rate.

Unfortunately, only a couple of mites survived on the onion bulbs and therefore there were

no useable data for onion bulbs from this experiment. The onion bulbs seem to be overall a poor host since thrips larvae also had high mortality. Whereas no thrips larvae were found dead in the disc assay (unless drowned) several larvae were found dead on the onion bulbs. These findings are consistent with results from a previous laboratory study where only 15% of onion thrips eggs survived to pupal stage on onion bulbs at 21°C compared with 75% survival on leek leaves and 85% survival on onion leaves (Jamieson et al. 2012). Based on the low consumption observed on the onion discs and the reduction in consumption found on the plastic bulbs compared with the plastic discs, it would seem that consumption of onion thrips larvae on actual onion bulbs in storage, at least if damaged, would be low. One possible explanation is that the sulphurous volatiles released from damaged onion cells (Whitaker 1976), were toxic to the mites. Previous studies have shown that some of these volatiles can be highly toxic for a non-adapted insect species (Dugravot et al. 2004). Another influential factor could be that the environmental conditions in the plastic containers with the bulbs were detrimental to survival of *N. cucumeris*. However, although the environmental conditions would be different in stored onion bins, the much higher survival rate of *N. cucumeris* on the plastic bulbs ($\approx 65\%$) compared to onion bulbs ($\approx 25\%$) suggests that this alone cannot account for the high mortality of *N. cucumeris*.

The results of the present study indicate that predation of onion thrips with *N. cucumeris* in storage facilities may not be effective based on the low consumption rate and survival of mites on onion bulbs. However, it is not known whether the predatory mite would exhibit similar low predation and survival on undamaged bulbs. Additionally, the results from a previous study where fewer thrips were found per bulb in 600 kg bins treated with *N. cucumeris* (Baker 2007; Baker & Powis 2009) may have been due to factors other than direct predation (e.g. the presence of the mites could potentially have caused the thrips to disperse from the bins).

ACKNOWLEDGEMENTS

We wish to thank Libby Burgess and Jessica Dohmen-Vereijssen for reviewing this manuscript. This work was funded by The New Zealand Institute for Plant & Food Research Limited.

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