



Evaluation of *Neoseiulus cucumeris* and *Amblyseius swirskii* (Acari: Phytoseiidae) as biological control agents of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae) on pepper

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ABSTRACT

The invasive chilli thrips, *Scirtothrips dorsalis* Hood poses a significant risk to many food and ornamental crops in the Caribbean, Florida and Texas. We evaluated two species of phytoseiid mites as predators of *S. dorsalis*. In leaf disc assays, gravid females of *Neoseiulus cucumeris* and *Amblyseius swirskii* both fed on *S. dorsalis* at statistically similar rates. Larvae were the preferred prey for both species, consuming on average 2.7/day, compared with 1.1–1.7 adults/day in no choice tests. Adult thrips were rarely consumed in subsequent choice tests when larvae were also present. Mite fecundity was statistically similar for both species feeding on thrips larvae (≈ 1.3 eggs/day) but significantly less for *A. swirskii* restricted to a diet of adult thrips (0.5 eggs/day). In greenhouse tests with infested pepper plants, both mite species established and reduced thrips numbers significantly over 28 days following a single release (30 mites/plant). However, *A. swirskii* was the more effective predator, consistently maintaining thrips below 1 per terminal leaf, compared with up to 36 for *N. cucumeris* and 70 in control treatments. Similar results were obtained for plants maintained outside in the landscape, where *A. swirskii* continued to reproduce and control thrips up to 63 days post release.

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1. Introduction

Chilli thrips, *Scirtothrips dorsalis* Hood, is a recent invasive pest now established in the Caribbean and Florida (Hodges et al., 2005; Seal et al., 2006b). *S. dorsalis* is highly polyphagous, with over 100 recorded hosts from at least 40 different families (Mound and Palmer, 1981). Both larvae and adults attack all above ground parts of host plants, preferring the young leaves, buds and fruits (Venette and Davis, 2004). Feeding damage causes distortion and turns leaves bronze to black while heavily infested plants become stunted or dwarfed, and leaves with petioles detach from the stem, causing defoliation in some plants. Among the economically important hosts are banana, bean, cashew, castor, corn, citrus, cocoa, cotton, eggplant, grape, kiwi, litchi, longan, mango, melon, onion, passion fruit, peach, peanut, pepper, poplar, rose, sacara, soybean, strawberry, sweet potato, tea, tobacco, tomato, and wild yams (Venette and Davis, 2004). *S. dorsalis* apparently is capable of spreading tomato spotted wilt virus on peanut (Amin et al., 1981), peanut necrosis virus (PBNV) and peanut chlorotic fan virus (PCFV) (Campbell et al., 2005) and tobacco streak virus (TSV) (Rao et al., 2003).

The invasiveness of this species is of primary concern. Although only considered established in North America (Palm Beach County, Florida), in October 2005, by September 2006, *S. dorsalis* had been detected in 24 counties (Silagyi and Dixon, 2006). Based on 2008 surveys, *S. dorsalis* is now considered established in at least two Texas counties (Scott Ludwig, personal communication). Due to its broad host range and invasiveness, *S. dorsalis* poses a significant economic threat to US agriculture and trade. The potential geographic distribution in North America could extend from southern Florida north along the western coastal states to the Canadian border, as well as the entire Caribbean region (Meissner et al., 2005; Nietschke et al., 2008; Venette and Davis, 2004). According to a USDA/APHIS economic assessment in 2004, potential losses on 10 primary host crops and 18 secondary host crops could equal to \$3.8 billion annually (Garrett, 2004).

Although thrips are most commonly targeted with insecticide applications (Morse and Hoddle, 2006), information regarding chemical control of *S. dorsalis* is limited. Seal et al. (2006a) showed chlorfenapyr, spinosad and imidacloprid were relatively effective (e.g. $\geq 88\%$ reduction with respect to controls), although the performance of novaluron, abamectin, spiromesifen, cyfluthrin, methiocarb, and azadirachtin were inconsistent without weekly application. However exclusive reliance on chemical insecticides

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is not a sustainable option for *S. dorsalis*, due to high costs and need for repeated sprays, risks of pesticide resistance with rapidly reproducing thrips populations, and adverse effects on beneficial organisms and the environment (Herron et al., 2007; Jensen, 2000; Loughner et al., 2005; Morse and Hoddle, 2006). As of August 2008, Dow AgroSciences has temporarily suspended the use of spinosyn products in two Florida counties following reports of resistance in western flower thrips, *Frankliniella occidentalis* Pergande <http://pestalert.ifas.ufl.edu/dow- conserve.htm>.

There is an urgent need for effective biological control agents for *S. dorsalis*. The predatory mite *Neoseiulus cucumeris* Oudemans has been used against *F. occidentalis* in greenhouse vegetables and ornamentals for many years with some success (Jacobson et al., 2001; McMurtry and Croft, 1997; van Houten et al., 1995; Williams, 2001) although its use as a biological control agent under field conditions has received little attention. More recently, another phytoseiid *Amblyseius swirskii* (Athias-Henriot) has received interest as a biological control agent of whiteflies and thrips and has been commercially available since 2005. Although relatively little is known about the suitability of thrips as prey for *A. swirskii*, emerging studies suggest it provide superior control of *F. occidentalis* compared with *N. cucumeris* (Messelink et al., 2006; van Houten et al., 2005). The use of phytoseiid mites against *S. dorsalis* has not been reported. Moreover, few studies have compared different phytoseiids in field and landscape settings, where *S. dorsalis* and other thrips are major issues. Here we report on studies comparing *N. cucumeris* and *A. swirskii* for control of *S. dorsalis* in laboratory, greenhouse and landscape tests.

2. Materials and methods

2.1. Insect and mite cultures

Scirtothrips dorsalis were obtained from a greenhouse colony maintained on cotton (unknown variety) at MREC. Predatory mites were obtained from Koppert Biological Systems Inc. (Romulus, MI) and IPM Laboratories, Inc. (Locke, NY).

2.2. Leaf disc bioassay

We compared predation and oviposition rates of *N. cucumeris* and *A. swirskii* on a diet of *S. dorsalis*, using modification of the method described by van Houten et al. (1995). The bioassay arena consisted of a single 2 cm diameter leaf disc removed from a 3–4 week cotton plant floated upside down on saturated cotton wool inside a 9 cm Petri dish. In 'no choice' tests, either 15 second instar or 15 adult thrips ($\approx 80\%$ female) were added to each leaf disc using a fine paintbrush and a dissecting microscope. In 'choice' tests, 8 larval and 8 adult thrips were added to each disc. A single gravid female mite selected at random from the colony (unknown age) was immediately added to discs along with no mite controls. A small piece of bran placed in the middle of each disc served as a refuge and oviposition substrate and reduced problems of mites (especially *N. cucumeris*) exiting discs and drowning. Dishes were fitted with a screened lid and incubated at L16:D8, 26 ± 1 °C and 60–70% r.h. Dishes were inspected at 24 h intervals for 4 days for numbers of live and dead thrips and mite eggs. Mites were transferred to a new arena (containing a fresh leaf discs and thrips) daily and any discs with missing mites were not scored. There were 3 replicate dishes per treatment and the study was repeated 5 times for both choice and no choice tests.

2.3. Greenhouse and landscape tests

Predatory mites were evaluated against established thrips infestations in sweet bell peppers *Capiscum annum* L. 'California

Wonder'. For each test, 24 one month-old plants were transplanted into 3.8 or 7.6 liter pots (larger pots were used in landscape tests to reduce problems with plants wilting) and inoculated with 30 adult *S. dorsalis* ($\approx 80\%$ female). Plants were fertilized weekly with Miracle Grow 12–4–8 NPK (Scotts Co. LLC) and placed in individual cages (61 × 61 × 137 cm covered with nylon mesh, 28 threads/cm) in a greenhouse. Predators were released after 7 days, when F1 thrips larvae were beginning to emerge. Treatments were *A. swirskii*, *N. cucumeris* (30 adults per plant) and controls (no mites). To investigate environmental effects, half the plants in each treatment (i.e. 4) were maintained in greenhouse cages while the other four were placed outside in MREC's research grounds. As the potential for dispersal between adjacent plants could not be discounted in the latter case (i.e. non-independence), treatments were separated by at least 100 m (placed in secluded grassy areas in partial sun) and data from each group of plants used as an individual replicate.

Plants were assessed weekly for 28 days following mite release. Mite and thrips life stages were sampled *in situ* from the four most terminal leaves ≥ 3 cm; adult and larvae *S. dorsalis* are most abundant on top leaves of pepper plants (Seal et al., 2006b). Plant were also scored according to the thrips damage scale outline by Kumar et al. (1996), (0) no symptoms; (1) terminal 3–4 leaves showing tiny eruptions in interveinal area; (2) terminal 3–4 leaves showing upward curling along leaf margin (3) severe scarring of terminal and a few basal leaves, (4) stunted plants, leaves severely curled and leaf area greatly reduced, (5) plants with no leaves and only stem remaining. There were four plants per treatment and the study was repeated on 3 separate dates throughout June–October 2008. Shade temperature and relative humidity were monitored period using a Hobo H8 Pro Series loggers (Onset Corp; Pocasset, MA). Treatment locations were rotated in landscape tests to minimize environmental bias from the surroundings.

2.4. Data analysis

In leaf disc assays, rates of mite predation and oviposition were compared using two-way univariate analysis of variance (ANOVA) with means separated where appropriate through Tukey's HSD tests at $P < 0.05$. Data were normalized via $\log(n + 1)$ prior to analysis. In greenhouse and landscape tests, effects of treatments and time on numbers of thrips and mites were analyzed using two-way repeated measures ANOVA (using each test as a replicate, $n = 3$), with plant damage compared using a nonparametric Kruskal–Wallis test (SPSS for Windows v 15).

3. Results

3.1. Leaf disc bioassay

Both *N. cucumeris* and *A. swirskii* consumed larval and adult *S. dorsalis*, although there were differences among tests. In 'no choice' tests, two-way ANOVA revealed both mite treatment (including controls) and thrips life stage exposed were significant factors for thrips mortality with a significant interaction term ($F_{2,83} = 98.3$; $P < 0.0001$, $F_{1,83} = 31.1$; $P < 0.0001$ and $F_{2,83} = 8.5$; $P < 0.0005$, respectively). Posthoc comparisons revealed no differences between the two mite species, although both species killed more larvae compared with adult thrips (Table 1). When mites were offered a choice, larvae were highly preferred prey, with the number of dead adults in 'choice tests' similar to background (control) mortality. Again, there were no differences in predation rates between the two mite species in 'choice tests'. There were overall differences in mite fecundity, depending on the thrips diet provided ($F_{2,77} = 7.2$, $P < 0.001$) but no overall differences between

Table 1

Daily consumption of *S. dorsalis* by two phytoseiid mites fed either larvae or adult thrips (no choice) or both (choice) in leaf disc assays. Control represents natural mortality.

	No choice tests		Choice tests	
	Larvae	Adults	Larvae	Adults
Control	0.18 (0.06)Ab	0.17 (0.05)Ab	0.05 (0.04)Ab	0.12 (0.04)A
<i>N. cucumeris</i>	2.75 (0.24)Aa	1.65 (0.28)Ba	2.03 (0.21)Aa	0.26 (0.08)B
<i>A. swirskii</i>	2.73 (0.21)Aa	1.09 (0.19)Ba	1.89 (0.20)Aa	0.24 (0.07)B

Data are mean (SEM) dead thrips per day for 15 mites monitored over 4 days. Lowercase letters (if present) show differences (Tukey's HSD test at $P < 0.05$) between mite treatments for a given thrips diet (i.e. columns); capitals show preference of thrips diet for a given mite species (independent sample t -test at $P < 0.05$) (rows). Lack of letters indicates the one-way ANOVA was not-significant ($P > 0.05$). No choice and choice tests were analyzed separately.

mite species ($F_{1,77} = 1.9$, $P = 0.17$) nor their interaction ($F_{2,77} = 1.7$, $P = 0.19$) in two-way ANOVA. Nevertheless, posthoc comparisons suggested that *A. swirskii* only fed adult thrips laid fewer eggs than *N. cucumeris* over the 4 days observation period; although no differences in fecundity between mite species were observed when larvae were present as prey (Table 2). Data were pooled over time

Table 2

Daily oviposition rate for gravid mites fed different stages of *S. dorsalis* in leaf disc assays.

	Thrips diet		
	Larvae	Adults	Larvae + adults
<i>N. cucumeris</i>	1.25 (0.17)	0.98 (0.15)a	1.29 (0.19)
<i>A. swirskii</i>	1.33 (0.16)A	0.52 (0.10)Bb	1.11 (0.15)A

Data are mean (SEM) dead thrips per day for 15 mites monitored over 4 days. Lowercase letters (if present) show differences between mite species for a given diet (columns) (independent sample t -tests at $P < 0.05$); capitals show effects of diets for each mite species (rows) (Tukey's HSD test at $P < 0.05$). Lack of letters indicates the one-way ANOVA was not-significant ($P > 0.05$).

for all analyses, as test date did not enter any significant treatment \times time interactions ($P > 0.05$).

3.2. Greenhouse and landscape tests

Thrips numbers increased rapidly in control plants in the F1 larval generation, reaching ≈ 60 larvae per terminal leaf by day 14 in greenhouse tests, and ≈ 50 larvae per terminal leaf in landscape tests by day 21, when leaves started to turn brown (Fig. 1). Two-way repeated measures ANOVA revealed the total number of thrips were affected by both treatment with predatory mites ($F_{2,11} = 35.1$, $P < 0.0001$) but not location (i.e. greenhouse versus landscape) ($F_{1,11} = 2.5$, $P = 0.14$), the interaction was also not significant ($F_{2,11} = 0.9$, $P = 0.45$). Both mite species reduced thrips numbers significantly compared with controls, although *A. swirskii* was the more effective predator compared with *N. cucumeris* ($P < 0.005$ in repeated measure HSD tests), with total numbers of thrips never exceeding 1 per leaf compared with up to 36 for *N. cucumeris*. By 28 days, control plants were severely stunted with little new growth, this period corresponding with a sharp decline in thrips numbers in greenhouse tests. Analysis of plant damage ratings at 28 days also showed no effect of location (Kruskal–Wallis chi-squared = 0.01, $df = 1$, $P = 0.93$) but a highly significant effect of mite treatment (Kruskal–Wallis chi-squared = 14.8, $df = 2$, $P < 0.001$, pooled across tests) with mean thrips damage scores of 1.3 for *A. swirskii*, 3.5 for *N. cucumeris* and 4.4 for no mite controls (Fig. 2).

Both mite species persisted and reproduced on pepper plants, although *A. swirskii* was recorded more frequently overall compared with *N. cucumeris*, especially at the end of the study ($F_{1,8} = 5.8$, $P < 0.05$), (Fig. 3). Both mite species reproduced; 47% of sampled mites were immature ($n = 804$). No predators were observed in control plants. *Amblyseius swirskii* persisted on plants from one test that were maintained for a further 5 weeks in the landscape, providing long term thrips control (i.e. 0.5 ± 0.25 thrips/plant at 63 days post

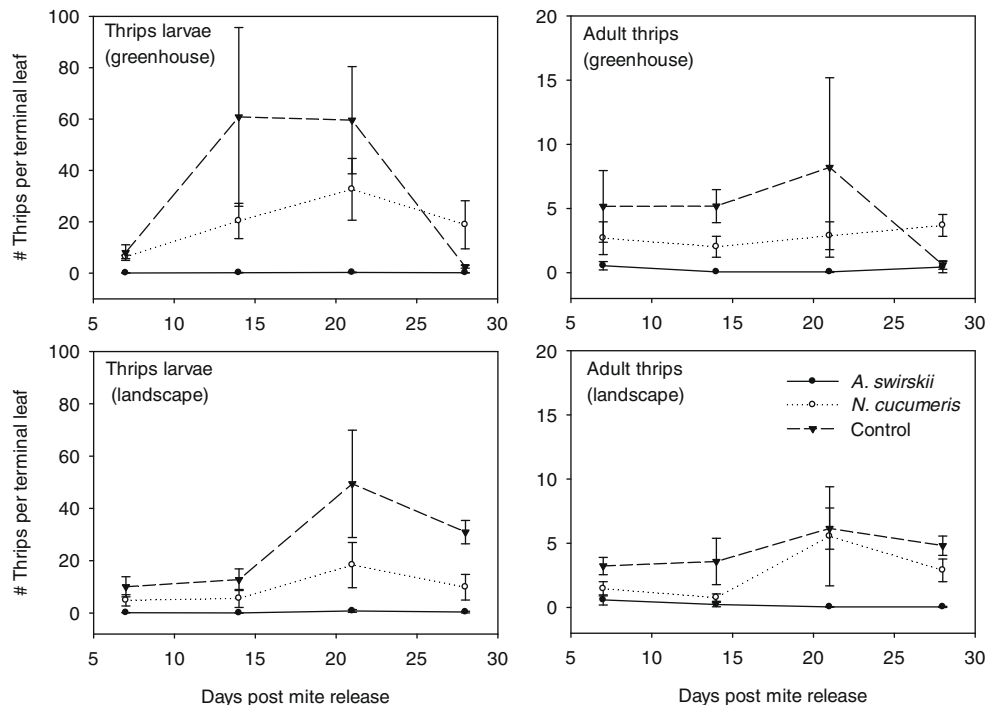


Fig. 1. *Scirtothrips dorsalis* populations (adults and larvae) on sweet pepper in greenhouse and landscape trials following a single release of two different predatory mite species (30 *N. cucumeris* or *A. swirskii*) as compared to an untreated control. Data show means \pm SEM from 3 separate tests.



Fig. 2. Greenhouse grown sweet pepper 35 days after infestation with *S. dorsalis* (30 adults per plant) and 28 days after release of predatory mites (30 adults per plant).

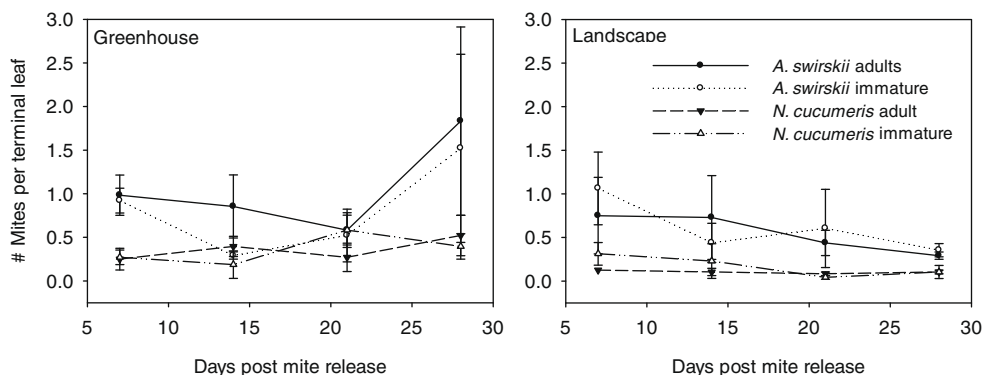


Fig. 3. Recovery of phytoseiid mites (adults and immature stages of *N. cucumeris* or *A. swirskii*) on thrips-infested sweet pepper in greenhouse and landscape trials following release of 30 mites per plant. Data show means \pm SEM from 3 separate tests.

thrips infestation). These plants produced a mean of 17.8 ± 4.5 fruit per plant which weighed 364 ± 39.5 g.

There were differences in environmental conditions, which were slightly warmer and more humid overall in the greenhouse cages versus landscape; i.e. mean 28.7°C (range $17.5\text{--}41.5$) and 90.4% r.h. (range $30.9\text{--}100$) versus mean 26.4°C (range $14.5\text{--}39.2$) and 56.3% r.h. (range $2.4\text{--}100$), respectively. Heavy rainfall (64 cm total) occurred during all landscape tests.

4. Discussion

Our data show that *A. swirskii* is a highly effective predator of *S. dorsalis* in sweet pepper, with single mite releases maintaining low thrips populations throughout our tests. Especially encouraging was the observation that mites reproduced and persisted under more challenging landscape conditions (e.g. more variable temperatures and exposure to wind and rain) up to 63 days post release. *Neoseiulus cucumeris* also reduced *S. dorsalis* populations, but was

less effective compared with *A. swirskii* and did not prevent economic damage to plants. Seal et al. (2006b) estimated that economic damage to chilli peppers by *S. dorsalis* occurred at densities of 0.5–2 individuals (larvae or adults) per terminal leaf. Our findings with *S. dorsalis* agree with those of van Houten et al. (2005) in control of *F. occidentalis*, who reported that releases of *A. swirskii* (30 mites per plant) provided better establishment and superior thrips control on greenhouse grown sweet pepper over 6 weeks compared with *N. cucumeris* released at same rates.

We noted that *A. swirskii* did not consume more thrips or lay more eggs than *N. cucumeris* in leaf disc assays, suggesting that its superiority as a predator of *S. dorsalis* in our tests on pepper plants may be due to some other aspects of its biology, such as its searching behavior. Leaf discs represent a highly simplified environment that does not reflect the complexity of the whole plant surface. Different environmental conditions between the tests may have also affected the two mite species differently. Native to the Mediterranean (Moraes de et al., 2004), *A. swirskii* may have also been better able to tolerate

the high daytime summer temperatures in our greenhouse/landscape studies ($\approx 40^\circ\text{C}$), which *N. cucumeris* is not well adapted for (van Houten et al., 2005).

Previous authors suggest that phytoseiid mites are especially persistent on pepper and other plants that provide flowers and extra floral nectaries as alternate food sources (Shipp and Ramakers, 2004; van Rijn and Tanigoshi, 1999). Peppers also contain leaf domatia, minute pockets at intersections of mid rib and lateral veins, which can protect phytoseiid mites against adverse environmental conditions and against cannibalism (Ferreira et al., 2008). It will be important to determine whether *A. swirskii* can control *S. dorsalis* effectively on plants lacking these structures, such as cucumber and ornamentals such as rose, chrysanthemum and bedding plants. One strategy to improve biological control in these cases is the provision of alternate food to promote survival of predators during times of low or zero pest density (Wade et al., 2008; Wackers et al., 2007). Hoogerbrugge et al. (2008) reported that pollen and other artificial food sources increase *A. swirskii* populations on chrysanthemum. However, pollen may also act as a competitor for prey. In tests when prey was also present, Skirvin et al. (2007) found that supplemental pollen led to a 55% reduction in thrips predation by *N. cucumeris*. Pollen might also serve as a food source for the prey, leading to higher prey populations.

The use of two or more natural enemies has also been suggested as a strategy to improve biological control of pests in greenhouses (Chow et al., 2008). Our laboratory studies show that *A. swirskii* and *N. cucumeris* were less effective predators of adult *S. dorsalis*, especially when larvae are present. Combined use of predatory mites with larger predators that readily attack adult thrips might be expected to enhance biological control in some cases. Chow et al. (2008) studied the compatibility of the anthorid flower bug *Orius insidiosus* Say with another phytoseiid species, *Amblyseius degenerans* in greenhouse tests on roses. In this example, combined releases of both predators did not enhance control of *F. occidentalis* compared with *O. insidiosus* alone, because *O. insidiosus* tended to switch to the most abundant prey and thus was an intra-guild predator of *A. degenerans*. Buitenhuis et al. (2008) also reported intra-guild predation between *A. swirskii* and *N. cucumeris*, with both species feeding on immature stages of the other. In prey choice tests on leaf discs, *A. swirskii* even preferred immature *N. cucumeris* over *F. occidentalis* larvae suggesting combined use of these mites should be avoided (Buitenhuis et al., 2008). Studies with alternative commercially available biological control agents, such as *Hypoaspis* mites or entomopathogenic nematodes, to target pre-pupae and pupae in the soil where predatory mites were not observed, are warranted.

In conclusion *A. swirskii* is a promising tool for managing *S. dorsalis* on pepper, although optimal release rates per plant still need to be evaluated. Koppert have developed a slow release sachet 'Swirskii mite Plus' that can be hung in crops. We are also planning further studies with *A. swirskii* in ornamental plants, including the possible use of pepper as a type of banker plant for predators.

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