Comparison of the phytoseiid mites *Amblyseius swirskii* and *Amblydromalus limonicus* for biological control of chilli thrips, *Scirtothrips dorsalis* (Thysanoptera: Thripidae)

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Abstract

The chilli thrips, *Scirtothrips dorsalis* Hood, is a recently established pest in the USA and poses a serious risk to many economically important ornamental and food crops. In this study the biological control potential of the phytoseiid mites *Amblydromalus limonicus* (Garman and McGregor) and *Amblyseius swirskii* (Athias-Henriot) was compared by examining their predation and oviposition rates when fed different developmental stages of *S. dorsalis*. Gravid females were offered 10 individuals of either first instar, second instar, or adult *S. dorsalis* using a no-choice leaf disc bioassay and oviposition and predation rates were assessed daily for 2 and 3 days, respectively. There was no significant difference in predation and oviposition rates between mite species fed specific *S. dorsalis* life stages. There was, however, a significant effect of *S. dorsalis* life stage on the oviposition and predation rates observed for each mite species. The larval stage was the most preferred stage for both mite species, with *A. swirskii* consuming 4.6–6.3 and *A. limonicus* 4.8–6.4 individuals/day compared to only 1.6–1.7 adults/day consumed by both species. Female *A. swirskii* and *A. limonicus* laid 0.55–0.75 and 0.73 eggs/day on the two larval stages, respectively, compared to only 0.25–0.30 eggs/day observed for individuals feeding on adults. Although the results showed that the biological control potential of both mite species was similar, having an additional predator available that may be as effective as *A. swirskii*, a proven control agent against *S. dorsalis* in the field, warrants additional research into its potential utility.

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Introduction

The chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae), is an invasive pest represented by a cryptic species complex (Dickey et al. 2015) native to East and Southeast Asia that has become established in areas representing a broad global distribution (Seal et al. 2006; Kumar et al. 2013). By 2010 in the USA, *S. dorsalis* had become well-established in Florida and Texas (Silagyi and Dixon 2006) and recent interceptions suggest there is a risk of *S. dorsalis* gaining a foothold in Alabama, California, Georgia, Louisiana, and New York (Diffie and Srinivasan 2010; Kumar et al. 2011; Dickey et al. 2015). In Florida where *S. dorsalis* has been most studied, it has been shown to be highly polyphagous, feeding on at least 50 plant species (Seal and Kumar 2010; Kumar et al. 2012) including many economically important ornamental, vegetable, and fruit crops. *Scirtothrips dorsalis* larvae and adults feed primarily on new leaf foliage, which can lead to stunted growth, unmarketable fruit, and even death of the plant during severe infestations. In addition to physical damage to the plant via feeding, *S. dorsalis* is also a vector for at least nine tospoviruses including melon yellow spot virus and tobacco streak virus (Rao et al. 2003; Chiemsombat et al. 2008) further increasing its ability to damage impacted crops.

Traditional control of *S. dorsalis* around the world has relied heavily on chemical options (Chu et al. 2006; Seal et al. 2006); however, recent studies have also demonstrated the utility of biorational insecticides and entomopathogenic fungi for controlling *S. dorsalis* (Seal et al. 2007; Seal and Kumar 2010; Aristizábal et al. 2017; Kumar et al. 2017; Dale and Borden 2018). Due to the rapid generational turnover of *S. dorsalis*, which can reproduce both sexually and parthenogenically (Dev 1964), there is elevated risk of populations developing resistance to insecticides. This has already been demonstrated for some Indian populations of *S. dorsalis*, which have exhibited resistance to organochlorine, organophosphate, and carbamate insecticides (Reddy et al. 1992; Sridhar and Rani 2003). Recent *S. dorsalis* control tactics have focused more on the use of natural enemies as a means of biological control in order to help prevent the development of insecticidal resistance, which can lead to decreased economic input by growers.

The mite *Amblyseius swirskii* (Athias-Henriot) (Acari: Phytoseiidae) is a generalist predator and has been used in the biological control of many mites (Messelink et al. 2006, 2010; van Maanen et al. 2010) and insects including whiteflies (Nomikou et al. 2002; Hoogerbrugge et al. 2011) and thrips (Wimmer et al. 2008; Chow et al. 2010; Kakkar et al. 2016). Use of *A. swirskii* against *S. dorsalis* is currently the primary means of its biological control and has shown potential for managing *S. dorsalis* populations (Arthurs et al. 2009; Doğramaci et al. 2011). The direct release of *A. swirskii* onto *S. dorsalis* infested crops (Kumar et al. 2015) or preventative release using banker plants (Arthurs et al. 2009; Xiao et al. 2012; Avery et al. 2014) have both been shown to be effective control tactics. Feedback from growers suggests that *A. swirskii* may have difficulty establishing on some host plants of *S. dorsalis* however, such as roses, which may be due to having few leaf trichomes which promote oviposition. Developing alternative biological control tactics for use with these hosts is essential to provide growers the necessary tools to prevent damage by *S. dorsalis*, and there is increased need to assess the utility of *A. swirskii* for *S. dorsalis* control beyond a select few vegetable crops.

In late 2011 the phytoseiid predatory mite *Amblydromalus limonicus* (Garman and McGregor) became commercially available. Like *A. swirskii*, *A. limonicus* is a generalist predator and an effective biological control agent against whiteflies (Hoogerbrugge et al. 2011; Knapp et al. 2013; Lee and Zhang 2018), psyllids (Davidson et al. 2016; Patel and

Zhang 2017), thrips (van Houten et al. 1995; Knapp et al. 2013; Vervoort et al. 2017), broad mites (McMurtry et al. 1984), and to a lesser extent spider mites due to its reduced performance on eggs (McMurtry and Scriven 1965) and poor ability to deal with webbing (van Houten et al. 2008; Vangansbeke et al. 2014). When compared to other phytoseiid species, *A. limonicus* has exhibited superior predation and oviposition rates feeding on thrips (van Houten et al. 1995; Houten et al. 2016). In studies directly comparing overall levels of thrips suppression between *A. limonicus* and *A. swirskii*, overall levels of thrips control were shown to be higher for *A. limonicus* against western flower thrips, *Frankliniella occidentalis* (Pergande) (Messelink et al. 2006).

Previous studies have shown that *A. swirskii* prefers feeding on the first instar of many thrips species and may have difficulty preying on later stages, as seen for poinsettia thrips, *Echinothrips americanus* Morgan (Ghasemzadeh et al. 2017), melon thrips, *Thrips palmi* Lindeman (Cuthbertson et al. 2012), and *F. occidentalis* (Wimmer et al. 2008). Due to the relatively small size of *S. dorsalis* compared to other species, however, *A. swirskii* can feed on both the larval and adult stages, albeit at a reduced rate for the latter (Arthurs et al. 2009). There is evidence that *A. limonicus* may have the capacity to feed on a broader range of thrips stages (Ghasemzadeh et al. 2017; Lam et al. 2019) than many phytoseiid species and it has been marketed for this behavior (Limonica®, Koppert Biological Systems). The aggressiveness of *A. limonicus* against the second instar and adult thrips may allow it to achieve better control of *S. dorsalis* than *A. swirskii*. Given evidence in the literature that suggests *A. limonicus* may be a superior natural enemy, the overall goal of this study was to compare the baseline effectiveness of *A. swirskii* and *A. limonicus* in controlling *S. dorsalis* in order to enhance ongoing biological control programs.

Materials and methods

Arthropod colonies

Chilli thrips were obtained from wild populations collected from ornamental roses (*Rosa* L.) in Apopka, FL, USA. Greenhouse colonies were maintained on bell peppers (*Capsicum annum* L.) at the University of Florida's Mid-Florida Research and Education Center (MREC) in Apopka. Colonies were held in screened cages ($59 \times 59 \times 59$ cm) within greenhouses (27.8 ± 0.13 °C, $85 \pm 0.43\%$ RH, under a natural light regime) and provided with clean host plants weekly.

Commercially reared *A. swirskii* and *A. limonicus* used in experiments were obtained from Koppert Biological Systems (Romulus, MI, USA). Mite species were confined separately within the bottom halves of 15-cm-diameter Petri dishes containing a black 8×8 cm cardboard square (which had been dipped in paraffin and etched with 1 mm^2 screening) placed on top of stacks of wet cotton pads (Kumar et al. 2015). Petri dishes were filled partially with water to keep the cotton moist and provide mites with a source of water. A glass microscope slide was placed over 5–10 pieces of 1-cm-long fibers (75% jute, 25% polyester) to provide mites an oviposition substrate and microspace to hide. Mites were provided cattail pollen (*Typha* sp.) twice a week as food. Mature adult females were removed from the laboratory colonies at their initiation, so that newly mature and gravid females (11–12 days old) in the colonies could be identified to ensure that even-aged individuals were used for laboratory trials. Mite populations were maintained up to 2 weeks in the laboratory before being restarted.

Plants

The commercial hybrid bell pepper ('RPP24272' Rogers/Syngenta Seeds, Boise, ID, USA) was selected for insect colony maintenance and to supply leaf discs for experiments. Clean pepper plants were housed in screened cages in greenhouses at the conditions described above at MREC. Plants were initiated in seed trays containing Jolly Gardener Pro-Line C/20 Growing Mix (Old Castle Lawn & Garden, Atlanta, GA, USA) and transplanted into 15-cm-diameter pots 2 weeks post-germination and fertilized with 5 g of Osmocote Plus 15-9-12 (N-P-K) (Scotts-Sierra Horticultural Products, Marysville, OH, USA). Pepper plants were watered as needed (2–3 × per week) and fertilized with Peter's Professional 20-10-20 (N-P-K, 325 ppm) (Scotts-Sierra Horticultural Products) once a week. Pepper plants were utilized for experiments 45 days after germination (6–8 true leaves) and were free of pesticides.

Laboratory assays

Predation and oviposition rates of *A. swirskii* and *A. limonicus* fed on different stages of *S. dorsalis* were assessed under laboratory conditions. Oviposition rates were assessed as a meaningful surrogate metric for population growth rates of phytoseiid mites (Janssen and Sabelis 1992). The experiment had a total of nine treatments consisting of each of the predatory mite species provided either the L1, L2, or adult stage of *S. dorsalis*. Controls consisted of each *S. dorsalis* stage in the absence of predators to assess baseline mortality. Bioassay arenas consisted of small Petri dishes (4.7 cm diameter) arranged in a 3 × 3 array within a larger Petri dish filled partially with water to isolate dishes. Within each small Petri dish, a 2.5-cm-diameter pepper leaf disc was placed onto a cotton round saturated with water to confine the mites and *S. dorsalis* to the leaf disc. A piece of rice husk was placed into the center of the leaf discs as a refuge to help encourage mites to stay on the leaf disc and a 1 cm fiber was added as an oviposition substrate.

A total of 10 thrips of the desired stage were added to each leaf disc using a fine tip brush under a dissecting microscope. A single female of *A. swirskii* or *A. limonicus* at the start of their oviposition period (11–12 days old) were also placed into the arenas as described above and arenas were covered with lids containing holes covered in fine mesh screening for ventilation. The arenas were placed into environmental chambers (25 ± 2 °C, 75 ± 10% RH, and L16:D8 h), with the large Petri dishes containing the nine smaller Petri dishes serving as blocks to control for potential positional effects within the chambers. The number of dead/alive thrips and total number of mite eggs were assessed once every 24 h for 3 days. Predation rate data from all 3 days was utilized; however, oviposition rate data from the first day were omitted to reduce the potential effects of mites' prior food source (Sabelis 1990). Leaf discs were replaced with fresh ones daily containing new thrips. The experiment was repeated with four blocks in time, with five treatment replicates performed during each experimental block in time for a total of 20 replicates per treatment.

Statistical analysis

All data were analyzed using SAS v.9.4 statistical software (SAS Institute 2013). Predation rate and oviposition rate data were log(x + 1)-transformed to meet the assumptions of homogeneity of variance and normality. Data were analyzed using a repeated measurements two-way ANOVA, with predator treatment and thrips stage as fixed effects and

experimental subject and observation day as random effects in Proc MIXED. Any significant differences observed among treatments were analyzed by Tukey's honestly significant difference (HSD) posthoc tests ($\alpha = 0.05$).

Results

The analyses indicated that experimental blocks were not statistically different for predation rates ($F_{3,168} = 1.27$, $p = 0.29$) and oviposition rates ($F_{3,111} = 1.64$, $p = 0.18$), so the data were averaged by experimental blocks for each mite species and thrips stage (n = 4 for each species-stage combination).

Predation rates

All three *S. dorsalis* life stages examined were consumed by *A. limonicus* and *A. swirskii* and there was no evidence of a pattern in daily predation rates exhibited by either mite species. There was a significant interaction between mite species and *S. dorsalis* life stage on observed mortality ($F_{4,171} = 47.22$, $p < 0.0001$). This interaction, however, was due to the mortality patterns of the control differing from the mite treatments and had no influence on the interpretation of the results. There was no significant difference in overall *S. dorsalis* mortality rates between mite species observed, however overall *S. dorsalis* mortality was significantly higher in both the *A. limonicus* and *A. swirskii* treatments compared to the control (Fig. 1). There were no significant differences in the number of *S. dorsalis* consumed between mite species for either first or second stage larvae, as well as adults.

Oviposition rates

There was no significant interaction effect between mite species and *S. dorsalis* life stage ($F_{2,114} = 0.44$, $p = 0.65$), or mite species treatment effect observed on mean daily oviposition rates ($F_{1,114} = 0.94$, $p = 0.33$) (Fig. 2). There was, however, a significant effect of *S. dorsalis* life stage observed on mean ovipositional rates of mites during days 2 and 3 ($F_{2,114}$

Fig. 1 Mean ($\pm$ SEM) daily predation rates (days 1–3) of the phytoseiid mites *Amblydromalus limonicus* and *Amblyseius swirskii* provided with 10 of either first or second stage larvae or adults of *Scirtothrips dorsalis* using a leaf disc bioassay. The control represents baseline thrips mortality. Letter differences indicate significant differences in predation/mortality rates between *S. dorsalis* stages within each predator treatment (Tukey's HSD test: $p < 0.05$)
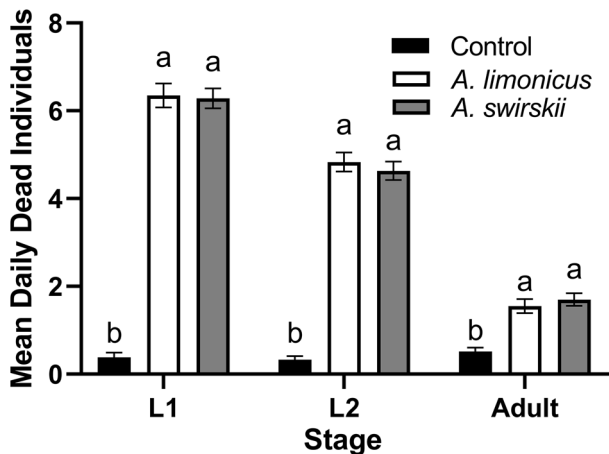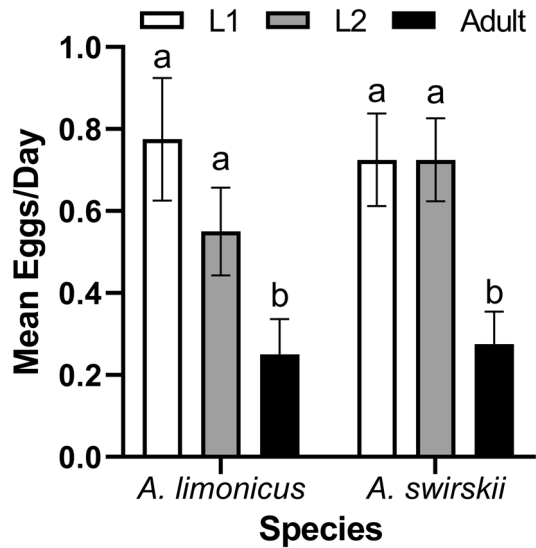
Fig. 2 Mean (± SEM) daily oviposition rates (days 2–3) of the phytoseiid mites *Amblydromalus limonicus* and *Amblyseius swirskii* provided with 10 of either first or second stage larvae or adults of *Scirtothrips dorsalis* using a leaf disc bioassay. Letter differences indicate differences in oviposition rates between mite treatments for each *S. dorsalis* life stage (Tukey's HSD test: $p < 0.05$)

$= 11.35$, $p < 0.0001$) (Fig. 2). Mean daily oviposition rates of mites fed on adult *S. dorsalis* were significantly lower than of individuals fed first and second stage larvae (Fig. 2). There was no difference in oviposition rates of mites fed first or second stage larvae (Fig. 2).

Discussion

Chilli thrips predation rates observed for *A. swirskii* observed in this study were slightly higher those observed by Arthurs et al. (2009) who found that *A. swirskii* consumed an average of 2.73 and 1.09 individuals/day of second stage larvae and adults respectively when provided 15 prey. Oviposition rates of *A. swirskii* observed in this study, however, were lower than the 1.33 and 0.52 eggs/day they observed for females fed second stage larvae and adults respectively. As in this study, a similar pattern of lower predation rates and fewer eggs laid by *A. swirskii* fed adult *S. dorsalis* was observed by Arthurs et al. (2009), suggesting adult *S. dorsalis* are a suboptimal prey. Our observations of *A. swirskii* predatory behavior in both the lab and field suggest that *A. swirskii* has difficulty subduing adult chili thrips to feed. In most encounters adult *S. dorsalis* drag mites across the leaf or kick them until the mites give up. However, in the field predation of adult thrips appears to be facilitated by leaf structures such as large veins and domatia that mites can use to pin the adult thrips against and successfully kill them. On rare occasion we have also observed additional *A. swirskii* adults, that were nearby *S. dorsalis* adults dragging pursuing adult mites, assist in successful predation attempts by jointly pinning down fleeing thrips. Both these observations suggest that adult *S. dorsalis* predation rates in the lab may underestimate predation rates of adults in the field.

As this was the first study to examine *A. limonicus* feeding on *S. dorsalis*, no direct comparisons of our findings with the literature can be made for this specific predator-prey combination; however, similar types of data exist for *A. limonicus* feeding on other thrips species. Ghasemzadeh et al. (2017) examined predation and oviposition rates on *E. americanus* by *A. limonicus* and *A. swirskii* and observed similar results, with no difference in

predation and oviposition rates of *A. limonicus* provided 10 first or second stage larvae. Unlike in this study, however, predation and oviposition rates of *A. limonicus* feeding on larvae were higher than those observed for *A. swirskii*. A study by van Houten et al. (1995) found that *A. limonicus* provided with 12 first instar *F. occidentalis* in a leaf disc bioassay consumed an average of 6.9 individuals/day and laid 3.2 eggs/day. Both studies saw considerably higher *A. limonicus* oviposition rates than what were observed in this study. Possible explanations for observed differences in these metrics may be attributed to population genetics of source populations of *A. limonicus* used as well as differences in the suitability of thrips species. It has been shown that *A. limonicus* performs well on plant material such as pollen and may also feed directly on plant tissue (Messelink et al. 2006). Studies examining *A. limonicus* oviposition rates on pollen saw higher oviposition rates than those observed in this study (van Houten et al. 1995; Vangansbeke et al. 2014; Leman and Messelink 2015; Nguyen et al. 2015; Samaras et al. 2015), and it would have been useful to include a pollen treatment as a reference to assess whether *S. dorsalis* is a poor food source.

In conclusion, results from this study show for the first time that *A. limonicus* is a promising candidate for use as a biological control agent against *S. dorsalis*. Although the hypothesis that *A. limonicus* would exhibit higher predation and oviposition rates than *A. swirskii* (especially on later developmental stages) was not supported by the data, the results still suggest that *A. limonicus* may be an equally effective predator. As *A. swirskii* has already been shown to provide effective control of *S. dorsalis* on some hosts such as pepper, there is the possibility that *A. limonicus* may possess other traits which could allow it to perform better in systems where *A. swirskii* is providing insufficient control. Documentation in the literature of greater thrips control achieved by *A. limonicus* compared to *A. swirskii* (e.g. Messelink et al. 2006; Hoogerbrugge et al. 2014) gives further credence to the hypothesis that behavior or biological traits other than predation and oviposition rates may better explain the effectiveness of *A. limonicus* as a biological control agent and needs to be examined in future studies.

Compliance with ethical standards

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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