Predatory Mite, Amblyseius swirskii (Acari: Phytoseiidae), for Biological Control of Asian Citrus Psyllid, Diaphorina citri (Hemiptera: Psyllidae)

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The Asian Citrus Psyllid (ACP), Diaphorina citri Kuwayama (Hemiptera: Psyllidae), is a serious pest of citrus in many citrus-producing regions. It vectors the bacterium Candidatus Liberibacter asiaticus’ thought to be the causal agent of the devastating “Huanglongbing” (HLB) or citrus greening disease. Both pest and the disease are well established in Florida. Several insect predators, particularly lady beetles and the parasitoid Tamarixia radiata (Waterston) (Hymenoptera: Eulophidae), are known to cause significant mortality to ACP immatures. However, there are no reports on the effectiveness of predatory mites against ACP. We evaluated the suitability of D. citri eggs and nymphs as prey for the predatory mite Amblyseius swirskii Athias-Henriot (Acari: Phytoseiidae) in laboratory arenas, and its potential to reduce psyllid populations in the glasshouse on caged Murraya paniculata (L.) Jack plants. Mortality of D. citri eggs on M. paniculata shoots exposed to A. swirskii in plastic arenas was 4 times greater after 6 d compared to unexposed control plants. Mites were also observed sucking out body fluids of first instar nymphs. In the glasshouse, total number of D. citri adults collected over 8 wk from infested plants in ventilated cylinders with A. swirskii present averaged 80% less than the control without mites. These findings showed a significant negative impact of A. swirskii on D. citri under controlled conditions. Further research needs to focus on rates and frequency of release, impact of A. swirskii on D. citri populations in citrus and other hosts under field conditions, and interactions of A. swirskii and D. citri with native predatory mites.

Key Words: Predation, Citrus sinensis, huanglongbing, phytoseiid mites, Murraya paniculata

Translation provided by the authors.
The Asian Citrus Psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) is an invasive pest of special concern, which has expanded its range throughout the citrus-producing regions of Asia and now the Americas. In the United States, the psyllid was first detected in Florida in Palm Beach, Broward and Martin Counties in June 1998 and quickly spread throughout the citrus-growing areas of the state (Halbert 1998; Halbert & Manjunath 2004; Michaud 2002; Tsai et al. 2002). It has also been identified in Texas, California, Arizona, and most of the south-eastern US (French et al. 2001, Qureshi & Stansly 2010). Hosts of *D. citri* are confined to Rutaceae, in particular the genus, Citrus and its relatives (Halbert & Manjunath 2004). The ornamental orange jasmine, *Murraya paniculata* (L.) Jack, a common hedge plant in south Florida, is considered a preferred host of ACP (Tsai et al. 2000). Direct injury to citrus by ACP results from phloem feeding on emerging foliage (flush), which causes permanent distortion or even abscession of new shoots with heavy infestation (Michaud 2004; Hall & Albrigo 2007). However, most economically important damage is caused by transmission of the bacterium *Candidatus Liberibacter asiaticus*, thought to be the causal agent of “huanglongbing” (HLB) or citrus greening disease (Halbert & Manjunath 2004). Symptoms indicating the presence of the bacterium are chlorosis resembling zinc deficiency, a more diagnostic blotchy or asymmetrical mottling of leaves, twig dieback with leaf and fruit drop, uneven coloring of fruits and reduction in fruit size and quality (Halbert & Manjunath 2004). Citrus greening disease was first detected in Florida in 2005 (Halbert 2005) and has spread throughout the state (http://www.freshfromflorida.com/pi/chp/ArcReader/mi2%20Sections%20in%20Florida%20Positive%20for%20HLB%2015%20Mile%20Buffer.pdf).

Integrated pest management (IPM) practices involving biological and chemical control strategies are being developed to suppress psyllid populations and to consequentially slow the spread of citrus greening (Qureshi & Stansly 2007, 2009, 2010). Native or exotic biological control agents of the psyllid include predators as diverse as ladybeetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysopidae), spiders (Aranae), and hoverflies (Diptera: Syrphidae) that together can greatly reduce the reproductive potential of the ACP population by more than 90% (Michaud 2002, 2004; Pluke et al. 2005; Qureshi & Stansly 2008, 2009). Two exotic parasitoids of ACP, *Dia- phorencyrtus aligarhensis* (Shafee, Alam and Agaral) (Hymenoptera: Encyrtidae) and *Tamarixia radiata* (Waterston) (Hymenoptera: Euphoridae) were introduced in Florida in 2000 against ACP (Hoy & Nguyen 2001). *Tamarixia radiata* is now widely distributed in the Florida citrus ecosystem at variable rates of parasitism (Qureshi et al. 2009) whereas *D. aligarhensis* has not established. Coccinellid predator species, *Olla v-nigrum* (Mulsant), *Curinus coeruleus* (Mulsant), *Harmonia axyridis* (Pallas), and *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae), are thought to be the most important sources of biotic mortality on *D. citri* nymphs in Florida (Michaud 2002, 2004; Michaud & Olsen 2004; Qureshi & Stansly 2009).

However, any single approach by itself is not going to provide enough reduction of the vector psyllid and citrus greening disease. Dormant season foliar sprays of broad spectrum insecticides in winter provide 5-27 fold reduction in ACP populations and opportunity for biological control in spring and summer (Qureshi & Stansly 2010). Nevertheless, the disease continues to advance, despite more frequent sprays of insecticides during growing season in many orchards.

This situation calls for a more proactive and augmentative approach to biological control, commencing with identification of other natural enemies of ACP. One avenue not yet investigated is biological control using predatory phytoseiid mites (Acari: Phytoseiidae) that could feed on the eggs and nymphs of *D. citri*. Phytoseiid mites are important agents of biological control on many pests in many crops. Depending on the species, their food may include mites, thrips and Hemiptera such as whiteflies and armored scales as well as pollen and honeydew (Dosse 1961; Putman 1962; McMurtry & Scriven 1964; Swirskii et al. 1967; Juan-Blasco et al. 2008). These findings have heightened interest in exploring additional possibilities for using mites to control different phytophagous organisms in many crops (Nomikou et al. 2001; Calvo et al. 2011). Thirty-eight species of phytoseiid mites have been reported in Florida citrus, although the biology of only a few has been studied (Abou-Setta & Childers 1987; Abou-Setta et al. 1997; Caceres & Childers 1991; Foulty et al. 1994; Yue et al. 1994). Much is yet to be learned about phytoseiids that colonize Florida citrus. We are aware of no published reports to date of any native or exotic phytoseiid attacking eggs or the first nymphal instar *D. citri*.

*Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) has shown itself to be a very efficient biological control agent of thrips [*Thrips tabaci* Lindeman and *Frankliniella occidentalis* (Pergande)] (Thysanoptera: Thripidae), whiteflies [*Triauleurodes vaporariorum* Westwood and *Bemisia tabaci* (Gennadius)] (Homoptera: Aleyrodidae) and phytophagous mites (*Tetranychus urticae* Koch (Acari: Tetranychidae) and *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae)) (Swirskii et al. 1967; Gerling et al. 2001; Nomikou et al. 2001; Calvo et al. 2011; Stansly & Castillo 2009, 2010). Eggs and crawlers (first instar nymphs) of *D. citri* are very similar in shape and size to those of *T. tabaci*. Eggs of *D. citri* are laid in the newly developing unfolded
leaves where they and the first instar nymphs are protected from large predators, but easily accessible to predatory mites. For the same reason, and due to its commercial availability and effectiveness as biological control agent of whiteflies and other pests in greenhouse, open field vegetable and citrus production (Stansly & Castillo 2009, 2010; Calvo et al. 2011; Juan Blasco et al. 2008), A. swirskii was selected for a preliminary evaluation against D. citri.

Our objectives were to determine whether 1) A. swirskii mites use D. citri eggs and nymphs as prey and 2) the mites suppress D. citri populations on young isolated M. paniculata plants following controlled release in a glasshouse.

MATERIALS AND METHODS

Cultures and Source Material

Diaphorina citri used in the experiments was obtained from a colony housed at the Southwest Florida Research and Education Center (SWFREC) in Immokalee, Florida, USA since 2005. The colony was maintained on M. paniculata plants propagated from seed and grown in a greenhouse covered with “Antivirus” insect netting in 15 or 20 cm pots using a substrate consisting of processed pine bark, 60% Canadian sphagnum peat and vermiculite. Plants were pruned 2 to 3 wk prior to use to induce emergence of new shoots before being moved to an air-conditioned glasshouse maintained at 27 ± 4 °C; 70 ± 10% RH. Six plants were placed in a wooden cage for oviposition twice a week (60 × 60 × 60 cm) with openings covered with fine polyester netting (40 × 10 mesh/cm) containing 300 adult ACP. After 3 d, plants were shaken and remaining adults vacuumed off. Plants were then transferred into an empty cage and held until emergence of adult ACP to be used for experimentation or to replenish the colony. Used plants were pruned, sprayed with a 2% glycerin soap solution (Clearly Natural Essentials Honeysuckle, Pure and Natural Glycerine Soap) and recycled for later use. Amblyseius swirskii sustained on the dried fruit mite Carpoglyphus lactis (L.) (Acari: Carpoglyphidae), was provided by Koppert Biological Systems (SWIRSKI-MITE®; Howell, Michigan, USA). Experiments were conducted from Nov 2007 to Feb 2008.

Laboratory Experiment

Predation by A. swirskii on eggs and nympha instars of D. citri was evaluated. Plants that had been pruned 8 d prior were placed in the oviposition cage with adult psyllids to infest new shoots as described above. A young shoot of M. paniculata infested with D. citri eggs was removed from a plant and placed inside an experimental arena consisting of a snap-cap polystyrene cylindrical vial (10 cm in length and 4 cm in diam). The bottom of the vial was removed and replaced by fine polyester organdy attached with hot glue. The polyethylene lid was perforated to receive a smaller plastic tube (length 1.7 cm, diam 0.7 cm) filled with water into which the stem of the shoot was inserted and sealed to the vial lid with plasticine to prevent escape of the mites. The vial was then inverted onto the cap to enclose the upper part of the shoot and placed in a metal grill over a plastic tray filled with water (Fig. 1).

Five A. swirskii adult females taken directly from SWIRSKI-MITE® bottles were placed in the experimental arena without alternative food. The experiment was set up the day SWIRSKI-MITE® bottles were received from manufacturer. Amblyseius swirskii females used in the experiment were neonate (maximum 2 days old) (Xu & Enkegaard 2010). There were 15 replicates of 3 treatments set out in a randomized complete block design: 1) shoots infested with D. citri eggs without A. swirskii, 2) A. swirskii adult mites with no D. citri or any other food source and 3) shoots infested with eggs of D. citri and A. swirskii adult mites. Vials were held in an air-conditioned rearing room maintained at 22 ± 2 °C, 63 ± 8% RH, 16:8 (L:D) photophase. Survival of A. swirskii adult females, the number of D. citri eggs and eventually D. citri nymphs were tallied under a stereoscopic microscope at intervals 0, 2, 4, and 6 d without replacement. Eggs and nymphs of D. citri were counted as dead when observed empty and desiccated.

Glasshouse experiment

The capacity of A. swirskii to suppress populations of D. citri on isolated plants was evaluated in an air-conditioned glasshouse maintained at 27 ± 4 °C; 70 ± 10% RH. Murraya paniculata plants, each in a 15 cm diam pot and with 4-6 young shoots were used for the experiment. Shoots were infested with D. citri eggs which were counted with the aid of a magnifying head set (2.75 X). Each plant was placed in a ventilated cylindrical cage made from a sealed sheet of clear plastic film covered on top with coarse mesh organdy (Qureshi et al. 2009, Fig. 2). Amblyseius swirskii adults were released on 4 plants at ratio of 1:2 (A. swirskii adults: D. citri eggs) inside of 30 mL plastic cups attached to the plant branch with wire. The correct number of A. swirskii was estimated by using a mean of 10 mites/gram of substrate obtained by counting mites in 10 randomly selected one-gram samples of substrate under a stereoscopic microscope. The control treatment consisted of plants infested with D. citri eggs but no mites. Emerging D. citri adults were aspirated off the plants and counted weekly.
for 8 wk. Leaves were inspected on the plant using the magnifying headset and phytoseiid eggs, larvae, nymphs and adults were recorded for 6 to 8 wk or until no more *D. citri* adults had been seen on the plants for 2 consecutive weeks. Larvae of the phytoseiids are easily distinguished from nymphs and adults by the presence of three pairs of legs. Nymphs and adults have four pairs of legs. Nymphs molt through the different nymphal stages (protonymph and deutonymph) to adult and leave the corresponding exuviae (Abad-Moyano et al. 2009). The counted exuviae were removed from plants.

**Data Analysis**

A generalized linear mixed model (GLMM) (Breslow & Clayton 1993) was used to analyze treatment effects on *D. citri* eggs and nymphs and on survival of *A. swirskii* using PASW Statistics version 19.0.0 (IBM SPSS Inc., Chicago, Illinois, USA; www.spss.com) for the laboratory experiment. Treatments (*A. swirskii* present or absent) were included as fixed effects, and shoots observed on d 2, 4 and 6 were included as a random effect. Selection of the best model was based on the Akaike Information Criterion (AIC). This revealed that the Poisson distribution with a logarithmic link was most appropriate to analyze for treatment effects.

The reduction of *D. citri* numbers attributable to *A. swirskii* predation on caged plants in the glasshouse was calculated using the Henderson-Tilton formula (Henderson & Tilton 1955). ANOVA was used to compare *D. citri* adult emergence between the *A. swirskii* and control treatments. *Diaphorina citri* adult emergence was square root transformed (sqrt (x)) to correct for heterogeneity of variance.
RESULTS

Laboratory Experiment

Adults of *A. swirskii* were observed preying upon eggs and first instar nymphs of *D. citri* on *M. paniculata* shoots. The predatory mites were observed sucking out the body fluids of the nymphs which then looked dried and empty. More dead *D. citri* eggs were observed in the presence of *A. swirskii* than in its absence (GLMM: $F_{1,28} = 103.18, P < 0.001$) (Fig. 3A). The treatment effect on number of dead nymphs was not significant (GLMM: $F_{1,28} = 0.01, P = 0.935$). Honeydew excretions from nymphs decreased over the 6-d observation period, indicating failure of the shoots to provide complete nutrition after the first few d which may have contributed to *D. citri* nymphal mortality. However, fewer live nymphs were observed on *A. swirskii* treated shoots than on untreated shoots (GLMM: $F_{1,28} = 46.68, P < 0.001$) (Fig. 3B). This suggests a negative effect on the development of nymphs from eggs in the presence of *A. swirskii*. Indeed, fewer *D. citri* nymphs (dead + live) were observed on shoots with *A. swirskii* adults compared with shoots without mites after 6 days (GLMM: $F_{1,28} = 18.98, P < 0.001$) (Fig. 3C). Survival of adult *A. swirskii* mites on shoots infested with *D. citri* eggs and nymphs was not different from shoots without *D. citri* eggs and nymphs up to 6 d (GLMM: $F_{1,28} = 2.67, P = 0.114$), which averaged 72 ± 8% on shoots infested with *D. citri* eggs and nymphs and 56 ± 13% on shoots without *D. citri* eggs and nymphs. Mite reproduction was limited over the 6 d period. One larva and 3 nymphs of *A. swirskii* were recorded in replicates with access to *D. citri*, and only 1 larva in replicates without *D. citri*.

Glasshouse Experiment

Initial numbers of *D. citri* eggs observed on *A. swirskii* treated and untreated plants averaged (± SE) 247 ± 37 and 240 ± 40 per plant, respectively. The total number of psyllid adults collected from plants with *A. swirskii* averaged 42 ± 11 which was 80% less than 204 ± 31 collected from control plants without *A. swirskii* (ANOVA: $F_{1,7} = 28.79, P = 0.002$) (Fig. 4). Most psyllid adults emerged during the second and third wk of observation. Most *A. swirskii* individuals (egg to adult) were observed 2 wk after release when a mean of 52 ± 15 per plant out of the initial 124 ± 21 per plant was found. The mean number of *A. swirskii* observed per plant had decreased the third week (24 ± 14), and then during wk 4 and 5 showed the minimum number of all life stages of *A. swirskii* observed, 5 ± 2 and 5 ± 3, respectively. Per plant numbers of all life stages of *A. swirskii* increased during wk 6, 7 and 8, averaging between 3 ± 0.5 - 5 ± 0.8, 9 ± 1.1 - 17 ± 1.8, 14 ± 1.5 - 33 ± 4.6, and 66 ± 5.7 - 86 ± 7.4 for eggs, larvae, nymphs and adults, respectively, during 3 wk period.

DISCUSSION

We observed that *D. citri* eggs on young shoots were preyed upon by *A. swirskii* mites under laboratory conditions and numbers of dead psyllid eggs were greater in the presence of the mites. To our knowledge this is the first time that a phytophagous mite was recorded preying upon eggs of *D. citri*. Although, predation was also observed on first instar nymphs of *D. citri* (0-6 d in the laboratory experiment), there was no statistically significant difference in the number of dead nymphs between *A. swirskii* treated and control shoots, presumably due to low survivorship in untreat-
ed controls. Possibly, shoot quality declined over time and could not provide enough nutrition for nymphs as evidenced by reduced honeydew secretion and high control mortality. Removal of young shoots from plants may have inhibited photoassimilate transport from mature leaves to shoot apices where eggs were laid and newly hatched nymphs fed. In contrast, adult emergence was 85% from nymphs that developed on intact shoots of caged plants in the control treatment in glasshouse.

Availability of alternative prey as well as non-prey food sources such as pollen and insect-produced honeydew are often important for predator establishment and persistence in the crop (González-Fernández et al. 2009; Nomikou et al. 2003, 2010). Non-prey food sources not only provide water and nutrients to complement a diet consisting of prey, but sometimes allow for predator reproduction. Nomikou et al. (2003) observed that cattail pollen supported survival, develop-

Fig. 3A. Mean (± SE) number of Diaphorina citri dead eggs observed at 2, 4 and 6 days on infested shoots of Murraya paniculata that were exposed or not to 5 Amblyseius swirskii adult females. The days of evaluation are counted from the infestation of M. paniculata shoots during ~ 48 h with fresh eggs of D. citri. The initial number of D. citri eggs on each shoot averaged 79 ± 8 on the first day of the trial.

Fig. 3B. Mean (± SE) number of Diaphorina citri live nymphs observed at 2, 4 and 6 days on infested shoots of Murraya paniculata that were exposed or not to 5 Amblyseius swirskii adult females. The days of evaluation are counted from the infestation of M. paniculata shoots during ~ 48 h with fresh eggs of D. citri. The initial number of D. citri eggs on each shoot averaged 79 ± 8 on the first day of the trial.

Fig. 3C. Mean (± SE) number of Diaphorina citri total (dead + live) nymphs observed at 2, 4 and 6 days on infested shoots of Murraya paniculata that were exposed or not to 5 Amblyseius swirskii adult females. The days of evaluation are counted from the infestation of M. paniculata shoots during ~ 48 h with fresh eggs of D. citri. The initial number of D. citri eggs on each shoot averaged 79 ± 8 on the first day of the trial.

Fig. 4. Mean (± SE) number of Diaphorina citri adults collected from Murraya paniculata plants infested with eggs of D. citri and placed under ventilated cylinder cages inside a glasshouse. Treatment plants received Amblyseius swirskii at a ratio 1:2 (A. swirskii adult: D. citri egg) compared with control plants with no mite release. Adults were collected weekly.
ment and reproduction of the two phytoseiid species *Amblyseius swirskii* and *Euseius scutalis* Athias-Henriot. Similarly, honeydew from *B. tabaci* greatly increased survival of *E. scutalis*, and supported development to adulthood and a high rate of oviposition. In contrast, coccid-produced honeydew did not promote development or oviposition of *E. scutalis* or *Amblyseius swirskii*, indicating that honeydew from *B. tabaci* may be of higher quality than coccid-produced honeydew, at least for *E. scutalis* (Swirski et al. 1967). Survival of adult *Amblyseius swirskii* was high with or without pollen or honeydew from *B. tabaci* provided on cucumber leaves, but oviposition by adults and juvenile survival was very low on a diet of honeydew compared with pollen (Nomikou et al. 2003). However, others (Ragusa & Swirski 1977; Momen & El-Saway 1993) observed enhanced survival of *Amblyseius swirskii* on honeydew produced by *B. tabaci*. Therefore, psyllid honeydew could be used by predatory mites as an alternative food source while searching for prey in the field. Additionally, previous studies have demonstrated that other species of phytoseiid mites are able to feed on plant sap (Grafton-Cardwell & Ouyang 1996; McMurtry & Croft 1997). Further investigation is needed to determine the suitability of psyllid honeydew for *Amblyseius swirskii* as well as the role that plant sap can play in their survival.

Citrus pollen could also be a useful non-prey food source for *Amblyseius swirskii*. Villanueva & Childers (2004) found a positive relationship between the number of phytoseiids and pollen grains on grapefruit leaves during the period of citrus flowering at Lake Alfred, Florida. In addition to pollen and honeydew from psyllids, *Amblyseius swirskii*, a polyphagous predator, could also benefit from other potential preys that colonize citrus such as several species of mites and thrips. Mixed diets are sometimes better food than any single type of food (Messelink et al. 2008). Both *Amblyseius swirskii* and *Euseius scutalis* had higher oviposition rates on diets of spider mites and almond anthers than on either food alone (Swirski et al. 1967). *Amblyseius swirskii* demonstrated higher oviposition on a mixed diet of eriophyoid mites and castor bean pollen than on pollen alone (Ragusa & Swirski 1977).

The glasshouse experiment was conducted to test if predation by *Amblyseius swirskii* upon *D. citri* observed on individual shoots could also be observed on isolated plants. *Amblyseius swirskii* released at a 1:2 (*Amblyseius swirskii* adult: *D. citri* egg, 124:247) ratio reduced the *D. citri* population up to 85% compared with control plants. This result demonstrated the potential for psyllid control using *Amblyseius swirskii*. Nomikou et al. (2002) also showed suppression of *B. tabaci* on single plants of cucumber using *Amblyseius swirskii*. Stansly & Castillo (2009, 2010) observed significant suppression of broad mite *Polyphagotarsonemus latus* (Banks) in the field using *Amblyseius swirskii* on both pepper and eggplant. Also, eggplant receiving *Amblyseius swirskii* yielded significantly more fruit than untreated plants or even eggplants receiving sprays of the acaricide spiromesifen. In this study, the increase in number of immature *Amblyseius swirskii* 1 month after the initial release indicated that they were probably using psyllid immatures and honeydew to support reproduction along with the food mites present with the SWIRSKI-MITE® formulation.

In this study, the predator *Amblyseius swirskii* showed promise for biological control against *D. citri* show some promise, but further tests are necessary to determine its possible role in the management of *D. citri*. *Amblyseius swirskii* inoculations could be used to reduce psyllid populations on young plants in the nurseries and field and thus, be used as a preventive measure to reduce *D. citri* eggs (inoculum). Future research should be focused on appropriate host plants (e.g., varieties of citrus or citrus relatives), reproduction of *Amblyseius swirskii* on *D. citri* diet, its prey preference, rates and frequency of the releases, density dependent effects and impact on *D. citri* populations in the field. Other predators, particularly lady beetles and the parasitoid, *T. radiata*, are already well established in Florida citrus and known to cause significant mortality to psyllid populations in the citrus groves (Michaud 2004; Qureshi & Stansly 2009; Qureshi et al. 2009). If proved effective in the field, *Amblyseius swirskii* could be a useful addition to enhance natural mortality of *D. citri* through its impact on eggs and first instar nymphs which are protected in newly developing unopened leaves and difficult to reach by most predators. These early immature stages are not targeted by nympha parasitoids which prefer later instars (Chu & Chien 1991). Finally, the predatory role of native mites on *D. citri* as well as their interactions with *Amblyseius swirskii* should also be evaluated.

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