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EFFECT OF POLLENS OF VARIOUS ORNAMENTAL PEPPER CULTIVARS ON THE DEVELOPMENT AND REPRODUCTION OF *AMBLYSEIUS SWIRSKII* (ACARI: PHYTOSEIIDAE)

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ABSTRACT

The rationale behind the current study was to assess if the commercially available generalist phytoseiid mite, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), can survive and reproduce when fed on pollen from ornamental pepper (*Capiscum annum* L.; Solanales: Solanaceae). Results from this study will help in the selection of ornamental pepper cultivars as candidate banker plants for establishment of *A. swirskii* in ornamental nurseries. To determine the effect of different types of pepper pollen on survival and multiplication ability of mites; their longevity, daily rate of oviposition and total egg deposition was assessed. *Amblyseius swirskii* was reared on pollen collected from 4 ornamental pepper cultivars namely ‘Red Missile’, ‘Masquerade’, ‘Black Pearl’ and ‘Explosive Ember’. *Amblyseius swirskii* was able to survive, develop and oviposit when fed with pollens of all the 4 ornamental pepper cultivars and the 2 standard controls i.e., commercially available olive pollen *Olea europaea* L. (Lamiales: Oleaceae) and *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae). No significant differences were observed among the various diet treatments for the following biological parameters: mean duration from larvae to adult death (22.8-23.7 days), duration from larvae to eggs (11.8-12.5 days) and adult longevity (12.4-13.1 days). However, for mites fed on pollen the highest daily rate of oviposition (~1.07 eggs/female/day) and total oviposition (~10.23 eggs/female) was recorded on the ‘Red Missile’. Outcome of this study can boost the management strategies being used against *S. dorsalis* and other pest species affecting ornamental plants in Florida.

Key Words: chilli thrips, banker plants, phytoseiid mites, biological control

RESUMEN

La lógica detrás de este estudio fue evaluar si el ácaro fitoseído depredador generalista, *Amblyseius swirskii* (Athias - Henriot), disponible comercialmente, puede sobrevivir y reproducirse cuando se alimenta de polen de pimienta ornamental (*Capiscum annum* L.; Solanales: Solanaceae). Para determinar el efecto de polen sobre la sobrevivencia y la capacidad de multiplicación de los ácaros; se evaluó su longevidad, tasa de oviposición diaria y la deposición total de huevos. *Amblyseius swirskii* fue criado en el polen recolectado de los siguientes 4 cultivares de pimienta ornamental, ‘Misile Rojo’, ‘Masquerade’, ‘Perla Negro’ y ‘Ember Explosivo’ los cuales son candidatos de plantas reservorias potenciales en viveros ornamentales. *Amblyseius swirskii* fue capaz de sobrevivir, desarrollar y poner huevos en todos los polen probados de cultivares de pimienta ornamental y los 2 controles estándar (polen de olivo disponibles comercialmente y *Scirtothrips dorsalis* Hood). No se observaron diferencias significativas entre los diferentes tratamientos dietéticos para los siguientes parámetros biológicos: el promedio de longevidad (22.8 a 23.7 días), la duración de huevos a larvas (11.8 a 12.5 días) y la duración del estadio adulto (12.4 a 13.1 días). Sin embargo, para

los ácaros alimentados con polen, la mayor tasa de la oviposición diaria (~ 1.07 huevos/hembra/día) y de la oviposición total (~ 10.23 huevos/hembra) se registró en el 'Misile Rojo'. Los resultados de este estudio ayudarán en la selección de candidatos de cultivares de pimiento como plantas reservorias para el establecimiento de *A. swirskii* en viveros ornamentales y estos resultados pueden impulsar las estrategias de manejo que se utilizan contra *S. dorsalis* y otras especies plaga.

Palabras Clave: trips de chile, plantas reservorias, ácaros fitoseidos, control biológico

In the last 2 decades, integrated biological control has been practiced as an important component of pest management strategies. It is a method of suppressing arthropod pest populations using suitable natural enemies to gain an economic benefit by integrating various biological control approaches including classical, inundative and conservation (Gurr et al. 1998; Gurr & Wratten 1999). The banker plant strategy is an example of an integrated biological control approach, which involves combined aspects of augmentative and conservation biological control and habitat manipulation proposed as an efficient alternative to synthetic pesticides (Osborne & Barrett 2005; Huang et al. 2011; Xiao et al. 2011). Banker plants in the agro-ecosystem can provide long-term suppression of pest populations by providing ecological infrastructure for sustenance of a reproducing population of natural enemies (Frank 2010; Huang et al. 2011). Banker plants can provide nutrient supplements in the form of nectar or pollen- important for the natural enemies survival in the absence of prey, as well as a modified microhabitat (domatium) which can protect them against adverse abiotic conditions and secondary enemies or from insecticide application (Landis et al. 2000). Such a self-sustaining pest management system can increase reliability of biological control strategies and can help reduce overall insecticide use. However, screening of plant varieties/cultivars to be used as banker plants is a complex step-wise process because host plants being used must possess certain qualities; 1) non-host or poor host of the pest, 2) ease of cultivation, 3) resistant to diseases, 4) adaptable to greenhouse conditions and 5) provide essential resources for survival and establishment of biocontrol agents (Huang et al. 2011). Thus, before selecting a plant species for use as a banker plant against a target pest, plant species must be screened for different parameters.

In the recent past, several researchers have reported *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) as an aggressive predator that can provide effective control against a number of vegetable or ornamental pests including tobacco whitefly, *Bemisia tabaci* Genn. (Hemiptera: Aleyrodidae); the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae); the western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae);

the chilli thrips *Scirtothrips dorsalis* Hood (Thysanoptera; Thripidae) and broad mites, *Polyphagotarsonemus latus* (Banks) (Arachnida: Acari: Tarsonemidae) in a timely fashion (Nomikou et al. 2001; Van Houten et al. 2005; Messelink et al. 2006; Arthurs et al. 2009; Xu and Enkegaard 2010; Dogramaci et al. 2011; Calvo et al. 2011). However, the survival and long-term establishment of the phytoseiid mites in the agroecosystem can be difficult in the absence of their prey. Scarcity of food may lead to cannibalism (Schausberger 2003; Momen & Abdel-Khalek 2009) resulting in a population crash of the phytoseiid mites, which can only be offset by multiple applications of mites making the strategy labor intensive and uneconomical. Currently *A. swirskii* is commercially available that can be released on the host plants in several different modes such as slow-release sachets, direct release of carriers (bran or vermiculite) on the leaves or substrate, or broadcast by air blast (Opit et al. 2005). However, none of these methods ensure longtime establishment of the phytoseiid mite in the agroecosystem. The goal of successful establishment of mites in the commercial production units of ornamentals and vegetables can be achieved by the use of banker plant which can support their population in multiple ways (characteristics mentioned above), even in the absence of their prey.

There are many studies which have shown that pollen produced by some of the plant species can provide nutrition and enhance phytoseiid mite survival and establishment, and suppress the pest population when required. Park et al. (2010) and Nomikou et al. (2010) showed that the biocontrol efficiency of *A. swirskii* against tomato russet mite *Aculops lycopersici* (Massee) and *B. tabaci* was improved when the predator was supplemented with the cattail pollen (*Typha latifolia* L.). In other related studies Van Rijn and Tanigoshi (1999a) and Ragusa et al. (2009) indicated that the absence of pollen led to decrease in egg deposition by the predatory mite population, *Iphiseius degenerans* (Berlese) and *Cydnodromus californicus* (McGregor), conversely significantly higher mite population was observed in the treatments where pollen was provided. Effect of different types of pollen on the development and reproductive ability of phytoseiid mites has also been reported. Van Rijn and Tanigoshi (1999b) tested 25 different types of pollen on life history param-

eters of two phytoseiid mite species *I. degenerans* and *Neoseiulus cucumeris* Oudemans and found that *I. degenerans* was able to utilize wider range of pollen for survival, however it had lower maximum ovipositional rate than *N. cucumeris*. In a recent study, Ranabhat et al. (2014) found 100% juvenile mortality of *N. cucumeris* in the two treatment groups where one received no food and the other group received castor bean (*Ricinus communis* L.) pollen. However, treatments provided with tulip and horse-chestnut pollen exhibited shortest development and the highest total fecundity of the mite species. All these studies suggest pollen of some selective plant species can facilitate the growth and development of phytoseiid mite species and mite species can be mass reared using these pollen types. However, the effect of pollen on the life history parameters can vary among different species of mites.

With the goal to develop a long-term management strategy against a highly polyphagous and emerging ornamental pest in the United States, *S. dorsalis* (Seal et al. 2010; Seal & Kumar 2010; Kumar et al. 2012, 2013, 2014), we conducted a series of studies to screen suitable banker plants which could support *A. swirskii* population in the absence of their prey. Ornamental pepper cultivars (as a banker plant for *A. swirskii*) were selected with the goal that these can be planted in rose nurseries, gardens and in the landscape infested with *S. dorsalis*. In previous studies (Xiao et al. 2012; Avery et al. 2014), we screened selected ornamental pepper (*Capiscum annum* L.; Solanales: Solanaceae) cultivars 'Masquerade' ('MA'), 'Red Missile' ('RM'), 'Black Pearl' ('BP') and 'Explosive Ember' ('EE') for some of the qualities of a banker plant such as growth and adaptability in the greenhouse condition, physical characteristics of plants important for mite establishment, and here we present a pilot study which was conducted to assess how efficient nutrient supplements these ornamental pepper can be for *A. swirskii* survival. Thus, the current study was designed to determine whether *A. swirskii* can develop and reproduce when fed solely on an ornamental pepper pollen diet. Evaluation of host pollen was an essential step (mentioned above) for screening of suitable banker plant cultivars.

MATERIALS AND METHODS

Pollen Collection

The study was conducted at the University of Florida's Indian River Research and Education Center, Fort Pierce, Florida (N 27.42° W 80.40°). Four cultivars of ornamental pepper (*Capiscum annum* L.) plants were grown from seed as single plants in Fafard Pro-mix medium (Conrad Fafard, Inc., Agawam, Massachusetts) in 10.1 cm-diam plastic pots and placed into a homemade plas-

tic screened cage (61 × 71 × 61 cm). Seeds of the 4 different ornamental pepper cultivars 'MA', 'RM', 'BP' and 'EE' (Ball Horticultural Co., West Chicago, Illinois, USA) were sown on different days to synchronize the germination times. Plants were irrigated as needed (~3 times a week) and fertilized with 50 mL/pot of Peters Professional® 20-10-20 (325 ppm) (Scotts Co., Marysville, Ohio) once a week. Two weeks after flowering, pollen was collected from flowers of each cultivar by tapping the pollen into small plastic ampoules. The collected pollen was dried in an oven for 2 days at 37 °C before storing in a refrigerator at 4 °C.

Stock Colony of *A. swirskii* Employed in the Experiment

The *A. swirskii* population used in the study was collected from a colony established at Mid-Florida Research and Education Center, Apopka, Florida (N 28.63° -W 81.55°) in 2009 from a culture of Swirski-Mite™ (Koppert Biological Systems., Inc. USA Ltd, Howell, Michigan). The mites were raised following a modified protocol of Carrillo et al. (2010) in plastic trays (14 × 14 cm) filled with water; the culture arena consisted of a waxed colored paper (5.5 × 5.5 cm) grooved by a wire mesh (1 mm²) placed on top of 3 stacked cotton pads (75 mm diam) with a few threads of cotton placed on top of the waxed paper simulating leaf trichomes (to facilitate egg oviposition of mites). Small dried apricots infested with all stages of *Carpoglyphus lactis* L. (Sarcoptiformes: Carpocephidae) were supplied to *A. swirskii* on plastic arenas as a food source. In order to produce mites of the same age for the current study, gravid female *A. swirskii* were kept individually in small Falcon® Petri dishes (4 cm diam) for 12-14 h to oviposit on leaf disks. The eggs were collected and incubated at 25 °C. The mites that emerged were considered to be of the same age or at least formed cohorts with a maximum age variation of less than 8 h and were used in the bioassay.

Feeding Experiment with *A. swirskii*

The experimental arena consisted of a small Falcon® Petri dish (4 cm diam) lined with a moist cotton layer and sealed with plastic food wrap. Plants selected for the leaf disk assay were healthy, young, vigorous, and free of arthropod pests. Leaf discs (3 cm diam) of each pepper cultivar ('RM', 'MA', 'EE' and 'BP') were punched at the center of the leaf blade beginning at the intersection between the petiole and the edge of the leaf with a cork borer and placed on the cotton. Between punches the tip of the borer was dipped in alcohol and flamed to prevent cross contamination of possible exudates between cultivars. About ~13-15 mg of pollen was provided on plastic cover slips and placed at the center of its respective plant's leaf disk. The cover slips, pollen and arena

were replaced every 3 days to avoid mold growth. For comparison, a commercial pollen source from an olive plant (*Olea europaea* L.; Lamiales: Oleaceae) (OC) (Pollen Collection and Sales, Inc. Lemon Cove, California) and *S. dorsalis* (SD) larvae were used as controls; these were put on 'MA' leaves as this cultivar was more leafy than other ornamental pepper cultivars. All the protocols used for control was similar to that of pepper pollen treatment. In SD treatment, 10 second instar larvae were provided in each Petri dish (instead of pollen) and dead preys were replaced every 24 h. Fifteen replicates were used for each treatment where each replicate consisted of a single newly emerged *A. swirskii* mite and were observed every 12 h to determine duration of immature stages in each treatment. Petri dishes were arranged in randomized complete block design and placed on a shelf in the growth chamber maintained at 25 ± 1 °C, 75 ± 5% RH and a 14 :10 h L:D photoperiod. Once development was complete, sex of the individual was confirmed and a male adult from the same cohort was added in each dish for a period of 48 h and replaced if needed. Adults (n = 15) were observed every 24 h until death using a binocular microscope (40X) and the number of eggs laid, oviposition and post-oviposition periods and longevity of *A. swirskii* were recorded for each treatment. In order to evaluate the effect of pollen on reproductive potential and avoid any cannibalism in *A. swirskii*, mite eggs were removed from the respective Petri dishes soon after recording. The entire study was repeated once.

Statistical Analysis

Data obtained on *A. swirskii* (egg numbers, and developmental period) during the study were analyzed independently using the Statistical Analysis System (SAS Institute Inc. 2003). When required, data were normalized using the square-root of (X + 0.25). The transformed data were then analyzed using one-way analysis of variance (ANOVA). Differences among the treatment means were tested using the Tukey's honestly significant difference (HSD) at α = 0.05. The non-transformed means are presented in the table and figure.

RESULTS

Amblyseius swirskii was able to survive, develop and oviposit after feeding exclusively on either pollen or *S. dorsalis* (SD) (Table 1). Duration of larvae to adult death of *A. swirskii* varied between 22.8-23.7 days when fed on different treatments (SD = 21-26 days; OC = 21-25 days; 'RM' = 21-25 days; 'MA' = 21-25 days; 'BP' = 20-25 days; 'EE' = 22-26 days), with no significant differences ($F = 2.38$; $df = 5, 174$; $P = 0.0603$) among

TABLE 1. BIOLOGICAL PARAMETERS (DEVELOPMENT AND REPRODUCTION) OF *AMBLYSEIUS SWIRSKII* FED ON SIX DIFFERENT FOOD SOURCES (FIVE POLLEN TYPES: RED MISSILE, MASQUARADE, BLACK PEARL, EXPLOSIVE EMBER AND OLIVE; AND THE THIRPS SCIRTOTHRIPS DORSALIS AT 25 ± 1 °C, 75 ± 5% RH AND 14:10 (L:D) H.

	<i>S. dorsalis</i> (SD)	Olive (OC)	'Red Missile' (RM)	'Masquerade' (MA)	'Explosive Ember' (EE)	'Black Pearl' (BP)
Total duration (larvae to adult death)	23.13 ± 0.25 a	22.96 ± 0.26 a	23.63 ± 0.21 a	23.26 ± 0.26 a	23.76 ± 0.24 a	22.83 ± 0.21 a
Larvae to eggs* (days)	11.80 ± 0.15 a	12.53 ± 0.38 a	12.53 ± 0.18 a	12.10 ± 0.14 a	12.36 ± 0.30 a	12.56 ± 0.24 a
Adult longevity (days)	13.03 ± 0.24 a	12.46 ± 0.26 a	13.10 ± 0.21 a	12.50 ± 0.26 a	13.16 ± 0.20 a	12.43 ± 0.26 a
Oviposition period (days)	11.56 ± 0.33 a	9.70 ± 0.51 ab	9.53 ± 0.29 ab	8.56 ± 0.55 b	9.70 ± 0.44 ab	9.20 ± 0.48 b
Post-oviposition period (days)	0.73 ± 0.21 c	1.20 ± 0.22 bc	2.03 ± 0.29 ab	2.96 ± 0.53 a	2.10 ± 0.29 ab	1.66 ± 0.28 abc
Total number of eggs	15.20 ± 0.66 a	9.86 ± 0.54 b	10.23 ± 0.56 b	8.50 ± 0.51 b	9.60 ± 0.55 b	8.70 ± 0.56 b
Daily rate of oviposition (eggs/day)	1.33 ± 0.06 a	1.10 ± 0.08 ab	1.07 ± 0.04 ab	1.05 ± 0.5 b	1.01 ± 0.05 b	1.02 ± 0.08 b

Means ± SEM (N = 15) within a row followed by the same letter are not significantly different ($P < 0.05$, Tukey's HSD).
*Days from larval emergence to first oviposition.

the means of different treatments. No significant differences were observed in other life cycle parameters including duration between larvae to first egg stage ~ 11.8 -12.5 days ($F = 1.57$; $df = 5, 174$; $P = 0.1707$) and adult longevity ~ 12.4 -13.1 days ($F = 2.21$; $df = 5, 174$; $P = 0.0551$) when phytoseiid mites fed on different food sources. However, the type of diet did influence reproductive potential of *A. swirskii*. A significantly higher number of *A. swirskii* eggs was recorded when mites were offered *S. dorsalis* as a food source (15.2 eggs/adult female) compared to the pollen treatments ($F = 19.37$; $df = 5, 174$; $P < .0001$). No significant difference was observed among pollen treatments, however numerically higher number of egg deposition was reported on 'RM' (10.2 eggs/adult female) followed by OC pollen (9.8 eggs/adult female). The daily oviposition rate of *A. swirskii* fed on *S. dorsalis* was significantly higher than 'MA', 'EE' and 'BP', but not from 'RM' and OC ($F = 3.60$; $df = 5, 174$; $P = 0.0040$). In all the treatments, oviposition rate was high during the 3-10 days of oviposition period (Fig. 1). The diet significantly influenced the duration of oviposition and post-oviposition period. *Amblyseius swirskii* fed with *S. dorsalis* had a significantly longer oviposition period than those fed 'MA', 'EE' and 'BP' pollen ($F = 5.20$; $df = 5, 174$; $P = 0.0002$) (Table 1). The longest duration of post-oviposition period was recorded for *A. swirskii* fed 'MA' pollen.

DISCUSSION

Biological control is an important component of integrated pest management (IPM) for any target pest species. Combining an inundative approach along with conservation biological control can aid the long term survival of biocontrol agents and result in achieving the goal of an IPM program- economically efficient strategy; which in turn can increase the acceptability of the management strategy. *Amblyseius swirskii* is a type III-b generalist predator which means it can feed on variety of food sources and like to live on glabrous leaves of the host plants (McMurtry & Croft 1997; McMurtry et al. 2013). Such ability of the phytoseiid mite species to develop on available alternative food makes them commercially viable and successful biocontrol agents.

There are number of studies available on life history parameters of different Phytoseiidae mite species on alternative nutrition source including pollen and nectar (Swirskii et al. 1967; Abou-Awad & Elsayi 1992; Fouly 1997; Van Rijn & Tanigoshi 1999a, b; Gnanvossou et al. 2003; Bouras & Papadoulis 2005; Papadopolous & Papadoulis 2008; Park et al. 2011; Onzo et al. 2012). However, there is little information available on the biological and reproductive parameters of *A. swirskii* on the ornamental pollen diets, especially ornamental pepper pollens. In this study, *A. swirskii* reproduced successfully after feeding only on

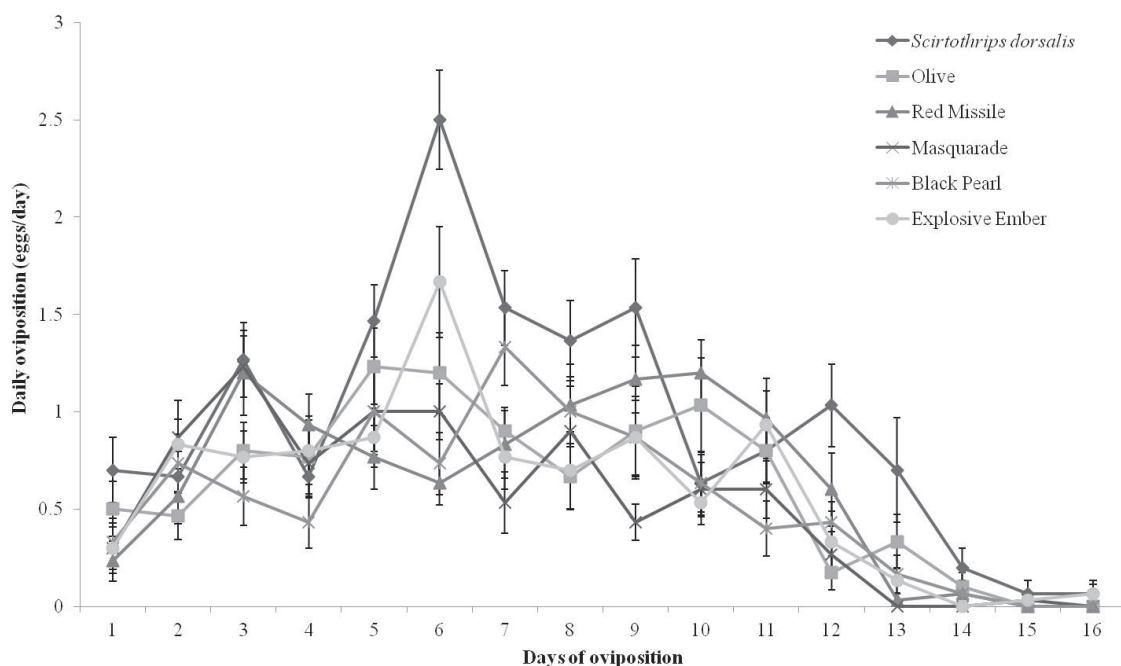


Fig. 1. Daily oviposition of *Amblyseius swirskii* females fed on either 'Red Missile', 'Masquerade', 'Black Pearl', 'Explosive Ember' and olive pollen or *Scirtothrips dorsalis* ($25 \pm 1^\circ\text{C}$, $75 \pm 5\%$ RH and a 14 h L: 10 h D). Error bars represent the standard error of the mean (Tukey's HSD, $P < 0.05$).

pollen from different ornamental pepper cultivars which demonstrates that this mite species can successfully survive and establish in nurseries even without the presence of prey. Results from the current study showed that both thrips and ornamental pollen diets were favorable in supporting survival, development and oviposition of *A. swirskii*. Among all the pepper pollen treatments, no significant difference in the various life parameters of *A. swirskii* was observed suggesting that all the 4 pepper cultivars were suitable for providing nutrient supplement to the predatory mite. The highest daily rate of oviposition and total number of eggs were recorded on 'RM', which concurs with the findings of Xiao et al. (2012) in a laboratory bioassay study that numerically higher number of eggs was reported in 'RM' compared to 'MA' and EE. However, in the current laboratory bioassay when the leaf discs were provisioned with the respective plant pollen we found mean number of eggs deposited per female was about 4 times higher than reported by Xiao et al. (2012). This suggests that the presence or absence of pollen can greatly influence the reproductive potential of *A. swirskii* and nutrition gained through a pollen diet can help in sustenance of mite populations in the absence of their prey. Our results are in confirmation with several other researchers (Swirskii et al. 1967; Van Rijn & Tanigoshi 1999b; Nomikou et al. 2003; Ranabhat et al. 2014) who found non-prey/pollen supplements can be utilized for survival and establishment (mass rearing) of phytoseiid mite species. However, different types of food supplement can have variable impacts on life history of phytoseiid mites.

Based on our findings, cultivar 'RM' along with other 3 potential banker plant candidates should be subjected to further screening as a potential ornamental banker plants for IPM of *S. dorsalis*. Because implementation and maintenance of the banker plants is easy and economical, and because phytoseiid mites are facultative pollen feeders, *A. swirskii* can potentially establish and provide suppression of multiple pests in ornamental nurseries. Our future studies will focus on further evaluation of these pepper cultivars for other biotic parameters including flower production, duration of flowering, life span, and effectiveness in protecting *A. swirskii* against chemical applications. Information on these biotic parameters will be important for their selection as a suitable banker plant for commercial nurseries.

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