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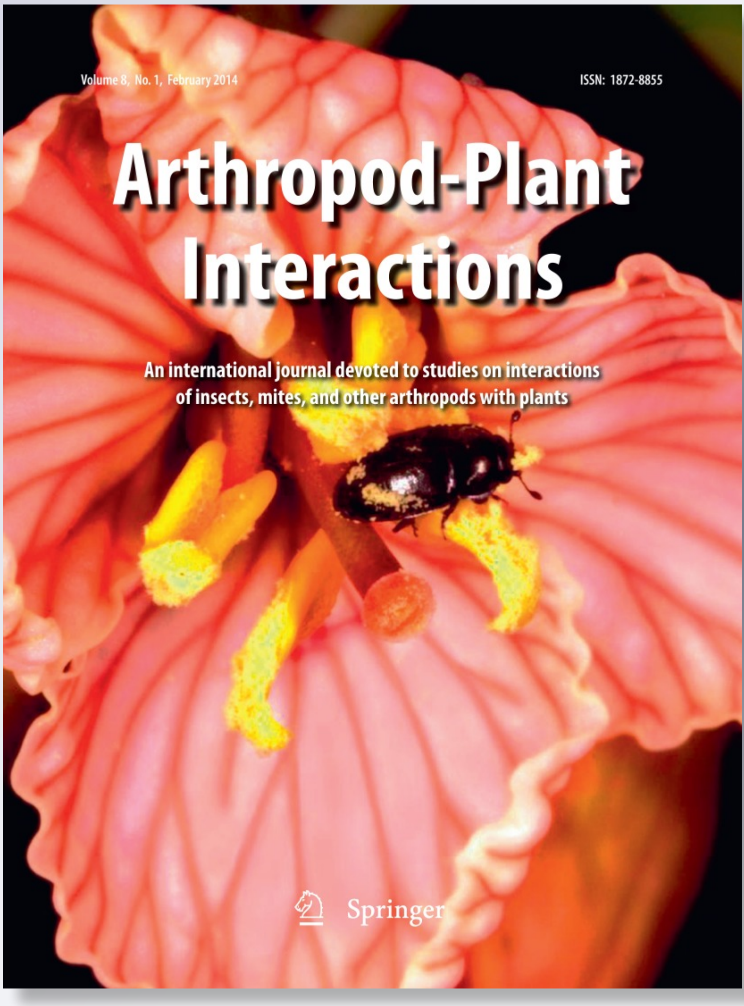

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ORIGINAL PAPER

Selecting an ornamental pepper banker plant for *Amblyseius swirskii* in floriculture crops

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Abstract Preference of phytoseiid mite, *Amblyseius swirskii* (Athias-Henriot) was assessed on four cultivars of ornamental pepper banker plant candidates; Red Missile (RM), Masquerade (MA), Explosive Ember (EE) and Black Pearl (BP) for potential control of pestiferous insects in floriculture. Significant differences in cultivar preference by *A. swirskii* was observed in choice experiments whether the test was pre- (with pollen) or during bloom. Overall, female mites laid more eggs when pollen was provided as a food source. The number of tuft domatia per cultivar leaf appeared to positively influence host preference in the choice plant tests pre-bloom. In addition, cultivar RM had the highest mean number ± SEM of tuft domatia per leaf (5.1 ± 0.3) and motiles per plant (4.0 ± 1.2), followed by MA, EE and BP. In choice tests on blooming plants, *A. swirskii* showed preference for both cultivars RM and MA compared to EE. These experiments indicated that the number of tuft domatia and availability of pollen can influence the host preference of *A. swirskii* for an ornamental pepper banker plant cultivar. Results from this study will help growers, researchers, educators and extension personnel in understanding the plant phenology promoting adoption of suitable banker plants for managing greenhouse and landscape insect pests.

Keywords Biological control · Tuft domatia · *Scirtothrips dorsalis* · Pepper banker plants · Pollen · Floriculture crops

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Introduction

Biological control has been widely used to manage many pests of vegetable and floriculture crops. A derivation of biological control is to explore the potential of banker plant systems in managing pests (Ramakers and Voet 1995; Osborne et al. 2005; Messelink et al. 2005, 2006; Jovicich et al. 2008; Frank 2010; Huang et al. 2011; Xiao et al. 2011a, b). Banker plant systems consist of a plant species that directly or indirectly provide resources, such as food, prey and shelter, to natural enemies that are deliberately released within a cropping system for suppressing the herbivorous pest population. Survival and establishment of natural enemies on banker plants is an advanced approach for long-term suppression of insect pests over augmentative biological control strategies which may require multiple releases of natural enemies over time.

A key factor supporting adoption of a successful banker plant candidate is the selection of plant species by predators with maximum likelihood of survival and establishment. It has been previously reported that plant phenology, e.g., the presence or absence of domatia with non-glandular trichomes in the vein axils (tuft domatia) plays an important role in the preference of host plants by predatory mites (O'Dowd and Willson 1991; Walter 1996). Tuft domatia can positively enhance predator numbers by: (1) functioning as a refugia for breeding and development, (2) reducing the chance that mites will be dislodged from the leaf surface, (3) increasing the capture of pollen or fungal spores which might serve as alternative food sources, (4) moderating the micro-environment, especially humidity, and (5) affording protection from intraguild predation (Walter and O'Dowd 1992a, b; Grostal and O'Dowd 1994; Walter 1996; Roda et al. 2000; Romero and Benson 2005). Romero and Benson (2005) indicated that the presence of domatia results in more beneficial mites on the leaves, fewer plant pathogen attacks and reduced leaf herbivory. When evaluating the influence of trichomes on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) abundance in grape varieties, Loughner et al. (2008) found that in general, a lack of trichomes was associated with much lower predator numbers and that phytoseiid abundance was best predicted by a model where domatia and hair (trichomes) had an additive effect. Loughner et al. (2010) later demonstrated using cotton fiber patches to mimic trichomes that the abundance of phytoseiid mites was positively associated with an abundance of non-glandular trichomes.

The predatory mite, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) has been shown to be an effective biological control agent against a number of pest species 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), all of which are commonly found damaging plants grown in greenhouses (Nomikou et al. 2001; van Houten et al. 2005; Messelink et al. 2006, 2008; Arthurs et al. 2009; Xu and Enkegaard 2010; Calvo et al. 2011). Commercially produced *A. swirskii* can be released by different methods, such as slow-release sachets (small breeding units that are hung on the plant and produce predators over several weeks), placed as piles in carriers (bran or vermiculite) on the leaves or substrate, or broadcast by air blast (Opit et al. 2005). However, a key issue for an effective biological control approach is to have predator populations established in a timely fashion to suppress targeted pests; otherwise, pesticide may be necessary to suppress pest populations below the economic threshold levels. Therefore, preventative releases on banker plants may provide a sustainable alternative for pest management as the predator can remain on these plants for long periods of time for suppressing pest infestations (Huang et al. 2011; Frank 2010). Once flowering, the banker plant can provide an alternative source of food (pollen) in the absence of their prey. Ragusa et al. (2009) demonstrated that the generalist phytoseiid, *Cydnodromus californicus* (McGregor) (Mesostigmata: Phytoseiidae) population increased when fed alternative food substances (pollen) present in the Mediterranean agroecosystems, and Nomikou et al. (2010) showed that the control of *B. tabaci* by *A. swirskii* can be improved by supplementing the predators with pollen. In their greenhouse study, Van Rijn et al. (2002) showed the addition of pollen to the host plant can increase the numbers of the phytoseiid mite, *A. degenerans* Berlese, resulting in greater reduction in thrips population.

Four ornamental pepper (*Capsicum annuum* L.) plant cultivars, Red Missile (RM), Masquerade (MA), Explosive Ember (EE) and Black Pearl (BP) were selected based on previous greenhouse studies conducted at the University of Florida, Mid-Research and Education Center in Apopka, FL, to screen the suitable banker plant candidates for the survival and establishment of the phytoseiid mite, *A. swirskii* (Xiao et al. 2012). Ornamental peppers were chosen because the original work was aimed at finding an ornamental plant that could be planted in rose gardens to foster biological control of *S. dorsalis*. *Scirtothrips dorsalis*, a highly polyphagous pest is a new invasive thrips species in the USA. Since its introduction in 2005, *S. dorsalis* has emerged as a significant pest of nursery, landscape ornamental and a few economically important field crops in the region (Seal and Kumar 2010; Seal et al. 2010; Kumar et al. 2012, 2013). Osborne (2009) has reported more than 50 different ornamental hosts of this pest in Florida. Use of chemical insecticide is considered a primary strategy for managing this pest. However, there are reports of resistance development in *S. dorsalis* to a wide range of chemical insecticides (Reddy et al. 1992; NPAG 2006; Kumar et al. 2013). Thus, it is important to devise an alternate pest management strategy for regulation of this species. In previous studies, we reported efficacy of *A. swirskii* in regulating *S. dorsalis* (Arthurs et al. 2009), and here, we chose to evaluate the factors supporting successful and long-term establishment of phytoseiid mites in the presence and absence of pest population with the use of banker plants.

Before evaluating pepper cultivars (RM, MA, EE and BP) in this study, cultivars were pre-screened and found to be a poor host of the target pest (*S. dorsalis*). Black Pearl was chosen for comparative purposes because it has been tested as a banker plant in the greenhouse previously with the predator, *Orius insidious* (Say) (Hemiptera: Anthocoridae) (Pundt and Smith 2008), but not with *A. swirskii*. The objective of this study was to compare the oviposition preference of *A. swirskii* with the physical characteristics of each specific ornamental pepper banker plant cultivar during pre-bloom and flowering stages of the host.

Materials and methods

Plant cultivars

The four ornamental pepper plant cultivars MA, RM, EE and BP were grown in Premier Pro-mix General Purpose Growing Medium from seed in seedling trays and placed into a plastic screened cage (61 cm × 71 cm × 61 cm). Plants in seedling trays were transplanted at different growth times into either 10 cm × 10 cm or 16 cm × 15.5 cm plastic pots depending on the experiment. Plants transplanted were placed back in screened plastic cages and watered as needed (~3 times a week) and fertilized with 50 mL/pot with Peter's Professional 20-10-20 (325 ppm) (Scotts Co., Marysville, OH) once a week at the ARS/USDA Horticultural Research Laboratory greenhouse, in Fort Pierce, FL. Temperature in the greenhouse ranged between 21.1 and 31.1 °C and the relative humidity from 42.0 to 99.5 %.

Rearing of *Amblyseius swirskii*

The predatory mites, *A. swirskii* obtained in paper sachets (SWIRSKI-MITE PLUS®, Koppert USA, Romulus, MI) were reared at 24–25 °C on the laboratory bench until needed. Each rearing unit/colony was as follows: 2 wax-covered colored paper squares (~55 mm × 55 mm) grooved by a wire mesh (1 mm^2) were placed on top of 3 stacked cotton rounds (75 mm diameter) and placed inside a plastic square container (140 mm × 140 mm). Water was added to each plastic square container to provide a moat to prevent escapes and to moisten the cotton pads. Fresh peach pollen (stored at 0 °C for <1 week) and cotton fibers (for oviposition) were placed on top of the upper most wax-covered paper square, and then gravid females (mixed ages) were released on the top square.

Choice experiments between plant cultivars pre-bloom with and without pollen

Four seeds (one from each cultivar) were randomly chosen and planted in a 10-cm diameter plastic pot using a template with all 4 cultivars equidistant from the center of the pot and each other. Plants were grown for 3–4 weeks (6–8 leaves/plant), and leaves of each cultivar were touching within the pot. A plastic Petri dish (60 × 15 mm) bottom containing peach pollen (13–15 mg) or no pollen (control) was placed in the center on the top of the soil between the plants (Fig. 1a). Four *A. swirskii* gravid female mites were gently removed from the rearing units using a moistened camel hair brush and transferred to 1 of 4 randomly chosen leaves/cultivar. Each mite was allowed to walk off the brush onto the leaf surface. This procedure was repeated 4 times/cultivar for a total of 16 mites released per pot, and there were 8 pots/experiment. Four out of 8 pots were randomly chosen and placed in separate plastic bowls set onto one of two separate trays in a randomized complete block design (Fig. 1b). Each tray was placed on a shelf in the growth chamber, and soapy water was poured into it as a moat to prevent escapes. All pots were held at 25 °C, 75 % RH under a 16-h L:8-h D photoperiod for 7 days. After 7 days post-release of the mites, the plants were destructively sampled and the number of *A. swirskii* life stages for each plant cultivar was assessed using a binocular microscope (40X). Four leaves were randomly chosen from each plant, and the number of tuft domatia was determined per leaf. This experiment was repeated on 2 separate dates.

Choice experiments between flowering plant cultivars

The assessment for preference by the *A. swirskii* mites on flowering plants was conducted only with three cultivars RM, MA and EE because bud formation in these cultivars occurred 28 days after planting (DAP), whereas with BP it took >68 DAP to flower. In preparation for the choice tests, each plant cultivar (RM, MA and EE) was grown in a seedling tray and placed in a screened plastic cage. Forty-five DAP, one plant of each cultivar was transplanted together into a single 3.78-L black plastic pot (16 cm × 15.5 cm), staked, twist tied and then placed back into the screened plastic cage. A plant from one cultivar was planted close enough in each pot in order to touch the leaves of the other two cultivars. Twelve pots with three plants each were transported and placed in the growth chamber (RH 75 %; 28 °C) at IRREC under a 12-h L:12-h D photoperiod until plants flowered. Each pot was placed in a plastic dish (20 cm × 7 cm) to hold water or fertilizer, and this dish was placed inside another dish (28 cm × 5 cm) filled with soapy water as a moat to prevent mite escapes (Fig. 1c). Five gravid female mites per plant cultivar were released on five different leaves per plant cultivar using a camel hair brush as described above; total of 15 mites were released per pot. Pots were watered 3× a week (150–200 mL) and fertilized 1× a week (200–250 mL/pot). After 28 days post-release, the plants were destructively sampled, placed in resealable plastic boxes (21 cm × 15.5 cm × 10 cm) and stored at 4 °C until assessed. The number of *A. swirskii* eggs, nymphs, adults for each plant cultivar/pot was assessed using a binocular scope (40×). This experiment was repeated on 2 separate dates.

Physical characteristics of plant cultivars

In a separate experiment, 100 leaves were randomly chosen from 10 plants/cultivar (75 DAP) and the number of tuft domatia was determined per leaf. The leaves were then photocopied, cut out using scissors and scanned using a

Fig. 1 **a** Arrangement of the four different ornamental pepper plants per pot for the choice test with and without pollen pre-bloom; **b** layout of the ornamental pepper pre-bloom choice tests in the growth chamber; **c** arrangement of the three different ornamental pepper flowering plants per pot for the choice test prior to being transferred to the growth chamber

LI-3100C Area Meter (LI-COR Biosciences, Lincoln, NE) to determine the surface area/cultivar leaf, and the number of tuft domatia/mm^2/leaf was calculated.

Statistical analysis

Preference of *A. swirskii* between plant cultivars (treatments) using choice tests pre- and during bloom was determined based on the mean number of life stages observed. An ANOVA was conducted on the square root ($n + 0.01$) transformed data with a mean separation between treatments using a LSD test ($P < 0.05$). The treatment effect of pollen (present or absent) in the amount of eggs deposited or motiles (nymph + adult) present on each cultivar was determined after conducting a t test ($P < 0.05$) in the pre-bloom studies. Also, in the pre-bloom studies, a correlation analysis was conducted using CORR PROC to determine whether there was any significant ($P < 0.05$) relationship between the number of tuft domatia/leaf and the number of eggs + motiles/plant cultivar when pollen was available. Significant differences in plant characteristics per cultivar: the number of leaves, flowers, tuft domatia/per mm^2/leaf was determined using an ANOVA ($\alpha = 0.05$) and LSD test on square root ($n + 0.01$) transformed data. Preference of *A. swirskii* for a banker plant cultivar during bloom was based on the difference in the mean number of eggs, nymphs, adults, and motiles present between cultivars. All statistical analyses were conducted using SAS Proc GLM procedures and executed on a WIN_PRO platform (SAS Version 9.2 2008).

Results

Choice experiments between plant cultivars pre-bloom with and without pollen

In the choice tests pre-bloom, the mean number of tuft domatia/leaf on RM was significantly higher ($F = 37.07$; $df = 3, 31$; $P < 0.001$) than the other cultivars (Table 1). The mean number of leaves/plant did not vary significantly among the cultivars. After 7 days post-release, when pollen was present, the mean number of motiles counted per plant was significantly higher ($F = 3.78$; $df = 3, 7$; $P = 0.0258$) for cultivar RM, whereas no significant difference was observed in motile counts among the other cultivars. No differences were apparent between cultivars when pollen was absent ($F = 0.03$; $df = 2, 7$; $P = 0.968$); however, no motiles were found on cultivar EE. When comparing the effect of pollen being present or absent on the same cultivar, a significantly higher mean number of motiles was found on cultivars RM ($t = -2.96$; $df = 1, 14$; $P = 0.0104$) and MA ($t = -2.21$; $df = 1, 14$; $P = 0.0444$)

Table 1 Plant characteristics and preference of *Amblyseius swirskii* motiles (nymphs and adults) for four different candidate banker plant ornamental pepper cultivars/pot with and without pollen in choice tests pre-bloom

Cultivar[b]	Mean number ± SEM[a]			
	Tuft domatia/leaf[c]	Leaves/plant	Motiles/plant	
			Pollen	Without pollen
RM	5.1 ± 0.3d	12.2 ± 0.5a	4.0 ± 1.2bB	0.4 ± 0.2A
MA	3.8 ± 0.3c	12.3 ± 0.7a	1.9 ± 1.0aB	0.4 ± 0.3A
EE	1.9 ± 0.3b	11.7 ± 1.0a	1.1 ± 0.4a	0
BP	1.0 ± 0.2a	12.5 ± 1.0a	0.8 ± 0.3aA	1.3 ± 1.3A

Four cultivars were planted in a single pot prior to releasing 4 *A. swirskii* on each. Plants were destructively sampled 7 days post-release ($n = 8$ pots)

[a] Data are square root ($n + 0.01$) transformed before analysis by ANOVA and LSD tests ($P < 0.05$). Untransformed data are presented. Mean numbers in a column with letters that are not the same are significantly different. Mean numbers with different capital letters across columns are significantly different (*t* test, $P < 0.05$)

[b] *RM* Red Missile, *MA* Masquerade, *EE* Explosive Ember, *BP* Black Pearl

[c] Results from 4 randomly selected leaves per plant/pot ($n = 32$ leaves/cultivar)

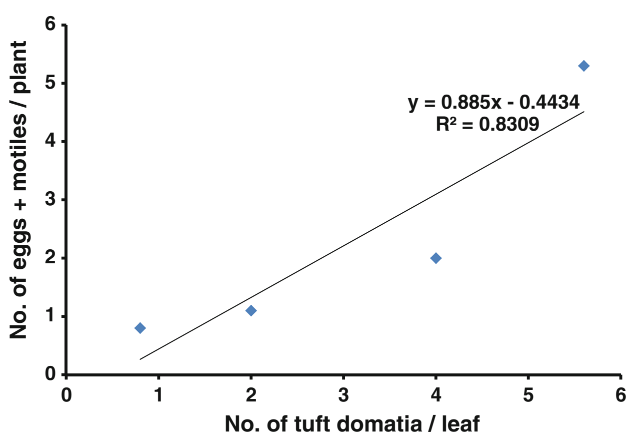

Fig. 2 Correlation between the number of tuft domatia/leaf and its effect on the number of *Amblyseius swirskii* eggs + motiles present pre-bloom for all the different ornamental pepper plant cultivars when incubated at 25 ± 0.5 °C with ~100 RH under a 16-h L:8-h D photoperiod regime. Each data point represents the mean number of eggs + mites (nymphs and adults) from 8 plants sampled per cultivar with pollen present

when pollen was present. The mean number of motiles recorded for cultivar BP was similar regardless of whether pollen was present or absent. A positive correlation ($R^2 = 0.8309$; $P = 0.0884$; $y = 0.885x - 0.4434$) was reported between the number of tuft domatia and the number of eggs + motiles/plant cultivar (Fig. 2).

Choice experiments between flowering plant cultivars

There were no significant differences in the mean number of eggs deposited by the gravid female *A. swirskii* mites among the different cultivars (Table 2). However, a significant preference was apparent for cultivars RM and MA in the mean number of nymphs ($F = 5.54$; $df = 2, 29$; $P = 0.0149$), adults ($F = 6.35$; $df = 2, 29$; $P = 0.0094$) and motiles ($F = 6.40$; $df = 2, 29$; $P = 0.0091$) per plant, compared to EE. The mean number of motiles/plant for cultivars RM, MA and EE was 314, 254 and 173, respectively.

Physical characteristics of different plant cultivars

The mean number of tuft domatia/leaf on RM was significantly higher ($F = 7.14$; $df = 2, 99$; $P = 0.0010$) compared to the other cultivars; however, the number per mm^2 was highest ($F = 20.30$; $df = 2, 99$; $P < 0.0001$) for EE (Table 2). The number of leaves/plant was significantly higher ($F = 4.10$; $df = 2, 9$; $P = 0.0342$) on RM than MA; however, the mean number of flowers/plant did not differ significantly among the different cultivars.

Discussion

We demonstrated in another similar experiment that after release of *A. swirskii* reared on the banker plants, they effectively suppressed both the population of *B. tabaci* and mixed thrips on green bean plants (Xiao et al. 2012). Such an effective suppression of these pests through this established banker plant system could provide a long-lasting and sustainable way of managing multiple pests under economically damaging levels in floriculture crops. Therefore, selection of the preferred ornamental pepper banker plant host(s) by *A. swirskii* was evaluated.

In the current study, female mites laid more eggs when pollen was provided as a food source in the pre-bloom studies. This finding is corroborated by other researchers concerning the effect of pollen on the mite reproductive capacity. Nomikou et al. (2010) and Park et al. (2010) indicated that the control of whiteflies and tomato russet mite by *A. swirskii* can be improved by supplementing the predators with pollen, respectively. In addition, van Rijn and Tanigoshi (1999) and Ragusa et al. (2009) found pollen sustains the reproduction and development of the predatory mite, *Iphiseius degenerans* and generalist mite, *Cydnodromus californicus*, respectively. Without pollen available as a supplementary food source in these studies, the number of eggs deposited decreased and the population of mites was significantly lower compared to those same cultivars when pollen was provided.

Table 2 Plant characteristics of different candidate banker plant ornamental pepper cultivars and mean number of *Amblyseius swirskii* life stages found on cultivars in choice tests during bloom 28 days post-release

Cultivar[b]	Mean number ± SEM[a]/							
	Leaf		Plant					
	Tuft domatia	Tuft domatia/mm^2	Leaves	Flowers	Eggs	Nymphs (n)	Adults (a)	Motiles (n + a)
RM	4.6 ± 0.2b	0.4 ± 0.1a[c]	198 ± 14b	27 ± 3a	51 ± 14a	129 ± 14b	185 ± 35b	314 ± 44b
MA	3.5 ± 0.2a	0.4 ± 0.1a	153 ± 12a	28 ± 3a	52 ± 14a	116 ± 14b	138 ± 29b	254 ± 39b
EE	3.9 ± 0.2a	0.8 ± 0.1b	180 ± 12ab	24 ± 5a	53 ± 14a	84 ± 17a	89 ± 17a	173 ± 32a

Three cultivars were transplanted in a single pot and allowed to flower prior to releasing 5 *A. swirskii* gravid females on each. Plants were destructively sampled ($n = 10$ pots)

[a] Data are log transformed before analysis by ANOVA and LSD tests ($P < 0.05$). Untransformed data are presented. Mean numbers in a column with letters that are not the same are significantly different

[b] *RM* Red Missile, *MA* Masquerade, *EE* Explosive Ember

[c] 10 leaves randomly chosen from 10 plants/cultivar at 40 days after planting ($n = 100$ leaves/cultivar)

In a concurrent study, when Kumar et al. (unpublished data) studied the effect of the pollen of four ornamental pepper cultivars (RM, MA, EE and BP) on development and reproduction of *A. swirskii*, they reported there was no significant difference in longevity and reproductive capacity of phytoseiid mite when fed solely on a pollen source. However, numerically, higher egg deposition was reported on treatment containing RM followed by MA, EE and BP. This result suggests that *A. swirskii* can successfully survive, develop and oviposit on all four ornamental pepper cultivars in the absence of prey when pollen is available as a source of nutrition. Therefore, an important aspect for selecting a particular cultivar as a suitable banker plant would be the time required for it to flower and produce pollen as an alternative source of food for the predator when being used early in the cropping season as a preventive measure for suppressing pest populations. In such a scenario, use of cultivars RM, MA and EE are preferred to cultivar BP which takes >68 DAP to flower, compared to 28 DAP for the other three cultivars.

In the current study, inside the domatia of the leaf, the non-glandular trichomes (tuft) present provided a place for the egg(s) to be attached and for the adult to inhabit. Some eggs were observed attached to the apex of the trichome, which extended several millimeters above the leaf surface or underneath several trichomes in or near the domatia. In the choice plant tests, pre-bloom between all four cultivars growing in a single pot supplemented with pollen and leaves touching, cultivar RM had the highest mean number of eggs deposited and tuft domatia per leaf, followed by MA, EE and lastly BP with the lowest egg numbers and least tuft domatia per leaf. The presence of tuft domatia has been shown to have a positive influence on the abundance of predatory phytoseiid mites (Pemberton and Turner 1989; Grostal and O'Dowd 1994; Agrawal 1997; Roda et al. 2000; Romero and Benson 2005; Loughner et al. 2008, 2010). Our results further support the idea that tuft domatia are preferred for increasing oviposition.

These experiments have indicated that the plant characteristics, especially the number of tuft domatia and availability of pollen as a supplemental food source, are some of the key aspects that can influence the selectivity and host preference of *A. swirskii* for an ornamental pepper banker plant cultivar. Based on our plant studies, *A. swirskii* demonstrated a preference for cultivar RM (pre-bloom) when supplemented with pollen and both RM and MA when flowering. In IPM programs, if the grower wants to utilize these ornamental pepper banker plants pre-bloom when no prey is available, it will be important to supplement *A. swirskii* released with some pollen, such as dusting on the leaves in order to sustain the population until plants begin to flower. Also, by growing all three cultivars in the same pot as in our study, using three RM or MA cultivars or a combination and then releasing the predatory mites during flowering, this strategy potentially may increase the carrying capacity of the banker plant system as well as the efficacy for suppressing the target pest. This hypothesis offers much promise, but needs to be tested under varying greenhouse and landscape conditions.

In conclusion, the three pepper varieties RM, MA and EE post-bloom, all supported a high number of the predator *A. swirskii* for suppressing populations of pestiferous insects in cropping systems. Further studies are needed to evaluate the dispersal and efficacy of *A. swirskii* on this banker plant cultivar system for managing various pestiferous arthropods found in floriculture production and landscapes.

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