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Early establishment of the phytoseiid mite *Amblyseius swirskii* (Acari: Phytoseiidae) on pepper seedlings in a Predator-in-First approach

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Abstract The establishment of biocontrol agents is critical for success of biological control strategies. Predator-in-First (PIF) is a prophylactic control strategy that aims to establish predators before the appearance of pests in an agro-ecosystem. PIF uses the ability of generalist phytoseiid mites to survive, develop and reproduce on pollen and thus establish in the absence of prey. The early establishment of populations of natural enemies helps control the pests at their incipient stage of infestation. The current study was undertaken to screen pepper cultivars for their ability to support populations of the predatory mite *Amblyseius swirskii* Athias–Henriot in the absence of prey. Twenty-nine pepper cultivars (11 hot and 18 sweet) were tested through a series of experiments, and four cultivars (7141, 992-7141, FPP7039 and FPP9048) were found to sustain *A. swirskii* populations throughout the study period. The initial application of pollen was important for establishment and maintenance of the predatory mites within the greenhouse system. Among the three screening experiments, high densities of mites were obtained in the experiment where 20 mites were released per plant, even reaching densities of >100 mites/plant. Recovery of predatory mites was significantly higher (ca. 2–3 fold) on the four pepper cultivars when predatory mites were mass released using an indirect method (banker plants) than when they were released directly on the seedlings, suggesting an advantage of passive continuous release. Future work will evaluate the selected pepper cultivars with the PIF strategy under greenhouse and field production conditions.

Keywords Banker plants · Prophylactic biological control · Predatory mites · Beneficials · Pest management

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Introduction

Since the early 1900s, when the first successful biological control result was reported against greenhouse whitefly (Speyer 1927), pest management professionals have worked continually to develop new ways to deliver consistent results with commercially produced natural enemies. Some of the strategies aiding establishment of beneficials in agro-ecosystems are: 'Pest-in-First'—the deliberate initial release of a selective pest (Luckmann and Metcalf 1975; Gonzalez and Wilson 1982; Messelink et al. 2008); 'Slow Release'—sachets consisting of beneficials along with a food source (Sampson 1998); 'Banker Plants'—alternative host plants providing food and shelter (Stary 1970; Parr and Stacey 1976; Bennison 1992; Frank 2010; Huang et al. 2011); and the use of artificial liquid food sprays as dietary supplements for predators (Wade et al. 2008). Although these conservation biological control strategies can support beneficial populations under certain circumstances, each has limitations that discourage widespread adoption. These may include, but are not limited to the: (1) release of non-pest insect species-growers are often reluctant to do so, (2) slow dispersal of biocontrol agents from the release point (sachets), (3) complexity in screening of non-host banker plants and their integration into multiple cropping systems, and (4) identification of chemical pesticides with minimal non-target effects (Stary 1993; Huang et al. 2011). Furthermore, conservation of natural enemies does not always result in effective suppression of pests under field conditions (Wade et al. 2008).

To overcome some of the aforementioned limitations of conservation biological control and to provide a tool for sustainable pest management, Ramakers (1990) suggested the concept of 'Predator-in-First' (PIF). The PIF approach aims to establish biological control agents in the critical seedling stage and/or early post-transplanting period when pest populations are absent. It uses the characteristics of generalist predators, which can survive and reproduce on plant-provisioned food sources in the absence of pest organisms (Ramakers 1995; McMurtry and Croft 1997; Nomikou et al. 2003, 2010; Park et al. 2010; Kumar et al. 2014a). Thus, PIF brings together the inundative and conservational biological control strategies where generalist phytoseiid mites released on vegetable seedlings or transplants are initially supported with plants with nutritional supplements in the early plant stages and then by flowers with pollen and/or pests in the later stages. The early establishment of natural enemy populations helps target the pests at their incipient stage of infestation.

Within the US, Florida is the second largest producer of fresh market vegetables after California, with 218,000 acres under production, valued at $1.9 billion (FDACS 2007). However, in recent years the Florida vegetable industry has been facing great challenges because of establishment of numerous invasive pest species. According to a report by the Florida Department of Agriculture and Consumer Services (2000), 150 species of exotic arthropods established in Florida between 1986 and 2000 (ca. 1 species/month), and some of these have become serious agricultural pests. Silverleaf whitefly [*Bemisia tabaci* (Gennadius)], western flower thrips [*Frankliniella occidentalis* Pergande], common blossom thrips [*Frankliniella schultzei* Trybom], melon thrips [*Thrips palmi* Karny] and chilli thrips [*Scirtothrips dorsalis* Hood] are of particular concern for the Florida vegetable industry (Demirozer et al. 2012; Kakkar et al. 2012; Seal et al. 2013; McKenzie et al. 2014; Kumar et al. 2013, 2014b). Apart from causing feeding damage to their hosts, these pest species can vector plant-damaging viruses and several are developing resistance against various chemical insecticides. Thus, to maintain an adequate supply of fresh produce and prevent the loss of agricultural jobs, it is important to address key issues affecting growers.

Table 1 Pepper cultivars evaluated for sustaining *Amblyseius swirskii* population in different studies

Pepper cultivar/ common name	Pepper type	Source	Study
7141	Bell pepper	Seminis Vegetable Seeds, Oxnard, CA, USA	Pollen study, sweet pepper screening, population dynamics, mite release method
992-7141[a]	Sweet bell pepper	Seminis	Pollen study, sweet pepper screening, population dynamics, mite release method
992-8302[a]	Sweet bell pepper	Seminis	Pollen study
994-2815[a]	Bell pepper	Seminis	Pollen study
997-9325	Sweet bell pepper	Seminis	Pollen study
FPP7039/Blitz	Sweet bell pepper	Sakata Seed America, Morgan Hill, CA, USA	Pollen study, sweet pepper screening, population dynamics, mite release method
FPP9048/Gridiron	Bell pepper	Sakata	Pollen study, sweet pepper screening, population dynamics, mite release method
SPP6001/Touchdown[a]	Sweet bell pepper	Sakata	Pollen study
Aristotle[a]	Sweet bell pepper	Seminis	Pollen study
Anaheim TMR	Hot pepper	Tomato Growers Supply (TGS), Fort Myers, FL, USA	Hot pepper screening
Bastille	Bell pepper	Syngenta Seeds Greensboro, NC, USA	Pollen study
Bayonet	Bell pepper	Syngenta	Pollen study
Big Chile Hybrid	Hot pepper	TGS	Hot pepper screening
Chilly Chili Hybrid	Ornamental, hot pepper	TGS	Hot pepper screening
Crusader[a]	Sweet bell pepper	Syngenta	Pollen study
Cutlass	Bell pepper	Syngenta	Pollen study
Explosive Ember	Ornamental, hot pepper	Ball Seed, West Chicago, IL, USA	Hot pepper screening
Fooled You Hybrid	Jalapeno pepper	TGS	Hot pepper screening
Hunter[a]	Sweet bell pepper	Syngenta	Pollen study, Sweet pepper screening
Intruder[a]	Sweet bell pepper	Syngenta	Pollen study
Jalapa Hybrid	Jalapeno pepper	TGS	Hot pepper screening
NuMex Sunburst Orange	Ornamental, hot pepper	TGS	Hot pepper screening
Rampart	Bell pepper	Syngenta	Pollen study
Red Missile	Ornamental pepper	Ball Seed	Pollen study
Riot	Hot pepper	TGS	Hot pepper screening
Super Chili Hybrid	Hot pepper	TGS	Hot pepper screening
Tam Jalapeno	Jalapeno pepper	TGS	Hot pepper screening

Table 1 continued

Pepper cultivar/ common name	Pepper type	Source	Study
Tom Cat[a]	Sweet bell pepper	Syngenta	Sweet pepper screening
Yellow Mushroom	Hot pepper	TGS	Hot pepper screening

At the time of conducting studies, some of the products were not in commercial production so both their product number and registered name have been included

[a] Commonly grown pepper cultivars in Florida (McAvoy and Ozores-Hamptom 2007)

Kutuk and Yigit (2011) found in their greenhouse study in Turkey that pre-establishment of *Amblyseius swirskii* Athias–Henriot on sweet pepper using *Pinus brutia* (Ten.) pollen provided effective reduction of *F. occidentalis* populations. However, except for a few greenhouse studies (Ramakers 1990; Kutuk and Yigit 2011), application of PIF has never been tested under field crop production conditions on pepper against its pests including *F. occidentalis*. In this study, we conducted a series of initial experiments to determine the effectiveness of the PIF for supporting phytoseiid mites under greenhouse conditions. The results of this study will provide a baseline for further tests of the PIF approach in supporting phytoseiids and regulating pest populations in commercial vegetable fields. We theorized that the PIF strategy for vegetable production systems could be sustainable because, following an initial release of mites, the vegetable seedlings themselves would harbor viable mite populations and multiple releases of mites would not be necessary. In addition, labor and chemical insecticide costs would be reduced, and the generalist predatory mites would attack multiple pests (thrips, whiteflies, broad mites, etc.) of vegetable crops (Nomikou et al. 2002; Messelink et al. 2008; Arthurs et al. 2009; Dogramaci et al. 2011; Calvo et al. 2011; Xiao et al. 2012).

As a primary step of the PIF approach, we screened several commercial pepper cultivars for their ability to sustain the phytoseiid mite *A. swirskii* in the absence of prey. The specific objectives of this study were to: (1) assess the effect of supplemental pollen on establishment of phytoseiid mites on pepper seedlings, (2) screen commercial pepper cultivars for their ability to sustain predatory mites in the absence of prey, and (3) evaluate rates and methods of mass inoculation of transplants with *A. swirskii* under greenhouse conditions.

Materials and methods

Pepper plants

All studies were conducted between 2011 and 2013 at the University of Florida's Mid-Florida Research and Education Center, Apopka (28.63N, 81.55W). The seeds of 29 commonly grown (11 hot and 18 sweet) pepper cultivars (Table 1) were sown on seedling trays containing Fafard 2-Mix growing medium (Conrad Fafard, Agawam, MA, USA) and when required, seedlings were transplanted into 10-cm-diameter plastic pots filled with the same growing medium in insect proof screen cages. Plants were watered as needed (ca. 3× a week) and fertilized with Peter's Professional 20-10-20 (325 ppm) (Scotts, Marysville, OH, USA) once a week. All seedlings were maintained in air-conditioned greenhouses

(27 ± 5 °C, 60 ± 10 % RH, L16:D8 h photoperiod). Plants selected for the studies were healthy, young, vigorous, and free of pests.

Laboratory rearing of predatory mites

Colonies of *A. swirskii* (Koppert Biological Systems, Howell, MI, USA), were reared on mixed pollens (peach and cattail) in 14 × 14 cm plastic trays isolated by water following a modified protocol of Carrillo et al. (2010). The culture arena consisted of a piece of black 5.5 × 5.5 cm cardstock dipped 2–3× in wax, and covered by a 1-mm^2 wire mesh square. Card stocks were placed on the top of three stacked 75-mm-diameter cotton pads with a few threads of cotton simulating leaf trichomes to facilitate oviposition. *A. swirskii* colonies were maintained this way for several generations before use in the bioassays. All rearing was conducted under laboratory conditions as mentioned above.

Effect of supplemental pollen on establishment of *Amblyseius swirskii* on pepper seedlings

To determine the importance of an initial application of pollen on the establishment of *A. swirskii* during the early stage of pepper growth, mite abundances on pepper seedlings were assessed in the presence and absence of supplemental pollen. Six seeds of a pepper cultivar were sown in small (3 × 2 cell) plastic trays as mentioned above. At 30 days post germination, two small cell trays containing four seedlings of uniform size, growth and vigor were selected and placed in separate cages (120 × 120 × 120 cm). One of the two cages was provided with a small amount (ca. 10–12 mg/seedling) of cattail pollen on the top leaves. Approximately 2 h after pollen application, five female *A. swirskii* adults were released on each seedling using a camel hair brush. All life stages of *A. swirskii* were recorded weekly for 4 weeks post-release on five leaves per plant. The experiment was conducted on 17 sweet pepper cultivars.

Screening of pepper cultivars

In order to screen pepper cultivars for their ability to sustain *A. swirskii* populations in the absence of prey, three separate experiments were conducted, using different rates of phytoseiid mite application. In the first experiment, the abundance of predatory mites was determined on 11 hot pepper cultivars with a low rate of initial mite (five per plant) release. For each cultivar, one potted seedling (5–7 leaf stage, non-flowering) was placed on an inverted small saucer (10 cm diameter) positioned in a larger saucer (20 cm diameter) filled with soapy water to prevent mites from escaping. The plants did not touch each other. Two weeks later, five adult female *A. swirskii* were brushed on the top leaves of each seedling and a small amount (ca. 10–12 mg) of cattail pollen was provided as a nutrition supplement. Eleven cultivars or species were used, each replicated 3× in a randomized complete block design. Starting 7 days after the release of *A. swirskii*, 15 leaves of each cultivar (5 leaves per plant × 3 plants) were non-destructively sampled weekly for 6 weeks. Life stages of *A. swirskii* were counted using a head-mounted 10× magnifier (Donegan Optical Company, Lenexa, KS, USA).

In a second experiment, eight sweet pepper cultivars were screened for their ability to sustain *A. swirskii* population in the absence of prey. The method of plant propagation and spatial arrangement for the experiment was similar to the above study except that it was conducted with a medium release rate of phytoseiid mites (10 per plant). Because the plants were not flowering at the beginning of the experiment, cattail pollen was added as a source of nutrition on the leaves of each seedling for the mites. Two hours after pollen application, ten adult females were released on each seedling. All stages of *A. swirskii* were recorded weekly on five top leaves of each pepper plant using a head-mounted 10× magnifier for a period of 8 weeks post release. Treatments were replicated 6× in a randomized complete block design.

Based on the results obtained in the previous experiment, we further screened four sweet pepper cultivars (7141, 992-7141, FPP 7039, and FPP-9048) to assess their ability to sustain a higher densities of *A. swirskii* without prey. The objective of the experiment was to assess if these cultivars had sufficient resources to support high mite densities in the absence of prey. The method of plant propagation, spatial arrangement for the experiment, and method of evaluation was similar to those in the above experiments, except that it was conducted with a high release rate of mites (20 per plant). The experiment had four treatments, replicated 6× in a randomized complete block design.

Effect of two release methods (banker vs. direct release) on densities of *Amblyseius swirskii*

This experiment served to evaluate two modes of release of phytoseiid mites: (a) direct application—mites released directly on the host plant, and (b) indirect application—mites released using banker plants in the treatment plots. The experiment utilized four sweet pepper cultivars (7141, 992-7141, FPP 7039, FPP-9048), and an ornamental pepper cultivar (Explosive Ember) as banker plants. Selection, preparation, and use of these banker plants under greenhouse conditions was described by Xiao et al. (2012). Each cultivar received *A. swirskii* through two release methods in the greenhouse. Seedlings were prepared and managed as described for previous experiments. For the banker plant release method, six potted (10 cm diameter) sweet pepper seedlings were placed on an isolated platform and one potted banker plant with a high and well-established population of *A. swirskii* was positioned so that its leaves touched those of the six sweet pepper plants enabling the mites to move between plants. In the direct-release treatment, six seedling pots were placed on an isolated platform without a banker plant. *A. swirskii* was released directly on each plant at the density of 20 female adults per plant. In order to compare the two release methods without any bias towards the direct-release method, pollen was not applied to the pepper plants. The populations of *A. swirskii* were visually checked on a weekly basis post-release for 6 weeks. During each sampling, five top leaves of each plant were examined using a head-mounted magnifier (10×). Each release method had four replications per cultivar with six seedlings per replicate.

Data analysis

Amblyseius swirskii data from the various experiments were analyzed independently using linear mixed model with the SAS procedure GLIMMIX with an autoregressive correlation structure (SAS Institute 2009). The model was used to determine the effect of plant cultivar, pollen/no pollen, and their interaction (effect of pollen experiment); cultivar, sampling week, and their interaction (screening of pepper cultivars); and cultivar, release method, and their interaction (mite release method), on *A. swirskii* numbers. The autoregressive correlation structure was applied to observations that were repeatedly measured each week to account for the correlation in data generated by re-sampling in time. The data were normalized using square root transformation to stabilize heterogeneous variance before analysis. When the interaction of treatment and time was found to be significant, mean separations were run only for differences in treatments in the same time period. The Tukey adjustment method was used because, as sets of comparison for a given time are orthogonal to each other, the total number of comparisons is greatly reduced, increasing the power to detect differences in the means. The effect of pollen or no pollen, and the two methods (banker vs. direct) of mite release, on mite density on each cultivar was tested using Student's *t* test. All tests were run at $\alpha = 0.05$. The data presented are the un-transformed means.

Results

Effect of supplemental pollen on establishment of *Amblyseius swirskii* on pepper seedling

There was a significant effect of pollen, cultivars and their interaction on mite densities (Table 2). When mite abundance on 17 sweet pepper cultivars was compared, a significantly high number of *A. swirskii* was reported in pollen-treated plots, and on cultivar FPP9048 (Tukey's test $P < 0.05$). Significant effects of the presence of pollen on predatory mite abundance were observed on nine pepper cultivars (Student's *t* test $P < 0.05$), where the presence of pollen positively influenced the predatory mite population on eight cultivars (Fig. 1). When pollen was supplied as a supplement, the highest mean densities of *A. swirskii* were found on FPP9048 and FPP7039 cultivars, whereas the lowest numbers were recorded on Crusader and Hunter. In the absence of pollen, low mite abundances were observed on all of the pepper cultivars throughout the study period. The highest mean density of *A. swirskii* was observed on 992-7141 followed by Crusader and the lowest

Table 2 ANOVA statistics for *Amblysieus swirskii* abundance in the three experiments

Experiment	Host	Effect	df[a]	*F*	*P*
Effect of pollen	Sweet pepper	Cultivar	16,120	7.94	<0.0001
		Pollen	1,193	30.09	<0.0001
		Cultivar × pollen	16,193	2.88	0.0003
Screening of pepper cultivars	Hot pepper	Cultivar	10,52	19.64	<0.0001
		Week	5, 106	65.63	<0.0001
		Cultivar × week	50,100	4.12	<0.0001
	Sweet pepper	Cultivar	7,128	22.37	<0.0001
		Week	7,270	75.12	<0.0001
		Cultivar × week	49,280	5.41	<0.0001
	Sweet pepper	Cultivar	3,46	31.85	<0.0001
		Week	6,112	54.24	<0.0001
		Cultivar × week	18,116	7.92	<0.0001
Mite release method	Sweet pepper	Cultivar	3,51	0.71	0.55
		Release method	1,64	74.67	<0.0001
		Cultivar × release method	3, 64	1.50	0.22

[a] All degrees of freedom were determined using the Kenward–Rogers method and decimals have been rounded to the nearest whole number

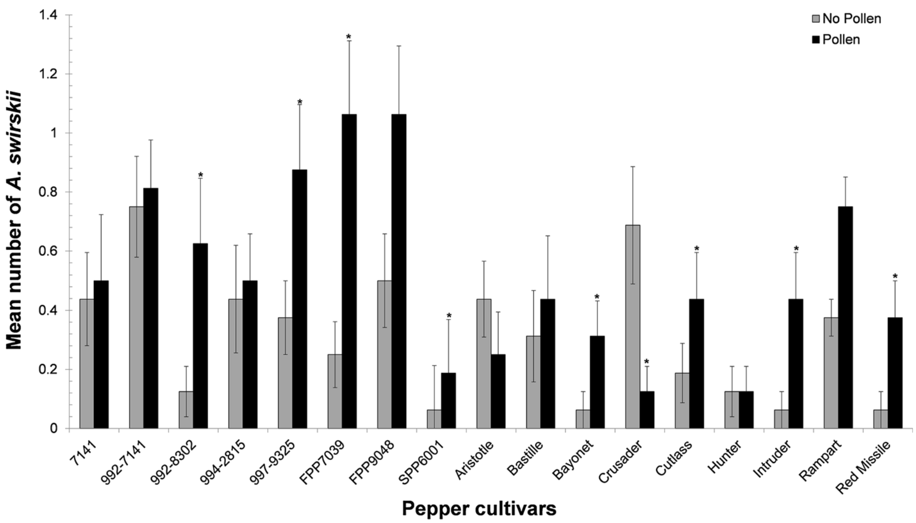

Fig. 1 The mean number (±SEM) of *Amblyseius swirskii* recorded per five leaves per seedling per cage in pollen and no-pollen treatments. *Asterisks* indicate significant differences in *A. swirskii* abundance on a pepper cultivar between pollen versus no-pollen treatments

densities were observed on Intruder, Bayonet, Red Missile and SPP 6001 (Fig. 1). Mites on two cultivars, Aristotle and Crusader, performed better with no pollen although mite numbers were significantly higher only on Crusader (Fig. 1).

Screening of pepper cultivars

In all three screening experiments, both the main effects (pepper cultivar and time) had a significant effect on the abundance of *A. swirskii* (Table 2). The effect of cultivars on the abundance of predatory mites on host plants varied over time, explaining the cultivar × week effects (Table 2). Weekly samplings showed overlapping generations of *A. swirskii* on hot pepper cultivars throughout the study period. In the first experiment, predatory mite populations were low at the beginning of study, and increased rapidly and peaked during week 3–4, gradually decreased at week 5 and then maintained low-moderate levels towards the end at week 6 (Table 3). Nevertheless, each cultivar of pepper sustained the predatory mite populations from seedling to the matured fruiting stage. High densities of *A. swirskii* on all cultivars were observed during the flowering period. A significant increase in predatory mite abundance compared to previous weeks was reported during the third sampling on all the pepper cultivars except for Riot, Yellow Mushroom, and Anaheim TMR (Tukey's test $P < 0.05$). Among hot pepper cultivars, a significant difference in predatory mite abundance was observed beginning at the second week (Table 3), where the highest density was observed on Fooled You Hybrid and the lowest on Anaheim TMR. From the third week onwards, a significantly higher abundance of predatory mites was reported on Chily Chili Hybrid than on Anaheim TMR, Numex Sunburst Orange (except week 3) and Riot on various sampling dates (Table 3). The highest mean number of predatory mites was recorded on Tam Jalapeno during week 4 followed by week 3, which were not significantly different from predatory mite densities on Chily Chili Hybrid and Explosive Ember on weeks 3 and 4, Yellow Mushroom on week 4, as well as from Super Chilli Hybrid and Fooled You Hybrid on week 3 (Tukey's test $P < 0.05$).

Table 3 Mean number (±SEM) of *Amblyseius swirskii* per five top leaves of hot pepper transplants on various sampling dates (weeks 1–6)

Pepper cultivars	1	2	3	4	5	6
Anaheim TMR	0.0 ± 0.0	0.33 ± 0.33b	2.0 ± 1.0c	1.0 ± 1.0d	0.66 ± 0.66 cd	0.0 ± 0.0d
Big Chile Hybrid	0.66 ± 0.33	2.0 ± 1.15ab	11.33 ± 3.48abc	5.66 ± 3.28bcd	1.0 ± 1.0 cd	3.0 ± 1.52abcd
Chilly Chili Hybrid	0.0 ± 0.0	3.66 ± 0.88ab	31.0 ± 5.68a	30.66 ± 4.63a	11.66 ± 2.18ab	6.0 ± 1.0a
Explosive Ember	0.0 ± 0.0	6.0 ± 1.52ab	29.33 ± 7.62a	22.66 ± 1.85ab	6.66 ± 2.33abcd	0.0 ± 0.0d
Fooled You Hybrid	0.0 ± 0.0	11.66 ± 3.84a	20.33 ± 3.52a	12.0 ± 2.31abcd	15.33 ± 1.66a	5.66 ± 1.76ab
Jalapa Hybrid	0.33 ± 0.33	7.66 ± 1.85ab	19.0 ± 6.65ab	20.0 ± 4.35abc	12.66 ± 4.09ab	3.66 ± 0.88abc
NuMex Sunburst Orange	0.0 ± 0.0	0.66 ± 0.66b	10.66 ± 4.66abc	1.0 ± 1.0d	2.0 ± 2.0bcd	0.0 ± 0.0d
Riot	1.0 ± 0.57	1.33 ± 0.33ab	1.0 ± 0.57c	2.33 ± 0.88 cd	0.33 ± 0.33d	0.66 ± 0.66 cd
Super Chilli Hybrid	0.0 ± 0.0	4.66 ± 3.71ab	21.66 ± 5.78a	9.0 ± 6.65bcd	10.66 ± 4.37abc	0.0 ± 0.0d
Tam Jalapeno	0.0 ± 0.0	2.66 ± 1.33ab	31.33 ± 4.48a	34.66 ± 2.16a	6.33 ± 1.45abcd	3.66 ± 0.66abc
Yellow Mushroom	3.33 ± 2.84	2.33 ± 0.33ab	3.33 ± 2.84bc	25.33 ± 9.35ab	4.33 ± 1.76abcd	1.66 ± 0.88bcd

Means within a column followed by the same letter are not significantly different (Tukey's test $P > 0.05$)

In the second experiment, sweet pepper cultivars sustained a low-moderate mite population throughout the study period. During weekly surveys, mites performed the best on cultivar 992-7141 maintaining a moderate-high level of mites between weeks 2 and 7, followed by 7141, FPP9048 and FPP7039 (Table 4). Significantly higher numbers of predatory mites were reported on 992-7141 than on Hunter and Cutlass on all sampling dates between weeks 4–7, TomCat on weeks 2 and 4–7 and Bayonet on weeks 2–4 and 6, respectively (Table 4). No significant difference in predatory mite abundance was reported between the cultivars FPP9048 and FPP7039 on any of the sampling dates, and between the 992-7141 and 7141 cultivars on different sampling dates except for week 4. A significant increase in predatory mite abundance compared to previous weeks was reported during the third sampling on cultivars 7141, FPP9048 and Cutlass (Tukey's test $P < 0.05$). A high density of *A. swirskii* was observed on all cultivars during the flowering period (weeks 3–6). The highest mean number of predatory mites was recorded on cultivar 992-7141 during week 5, which was not significantly different from densities on 7141 in week 5 and 992-7141 in week 4 (Tukey's test $P > 0.05$). The lowest mite density was reported on Bayonet cultivar during week 8.

In the third experiment, where 20 mites were released per plant, cultivar 7141 outperformed the remaining three cultivars in supporting populations of *A. swirskii*. Low-moderate densities of *A. swirskii* were observed on 7141, FPP7039 and FPP9048 during the first few weeks after transplant, which peaked to the highest level after week 4 and then decreased to low levels after week 6 (Fig. 2). Low densities of mites were observed on 992-7141 during the entire study period. *A. swirskii* was highest on 7141 in week 5 and lowest on FPP7039 in week 1 (Tukey's test $P < 0.05$).

Effect of two release methods (banker vs. direct release) on population abundance of *Amblyseius swirskii*

Abundance of *A. swirskii* was affected by the release method, but not by cultivars, nor by their interaction (Table 2). Except for a few occasions, consistently high mite numbers were found in the banker plant treatment compared to the direct-release treatment (Fig. 3). A significantly higher number of mites was found in the banker plant treatment than in the direct-release treatment for all the four cultivars (Student's t test $P < 0.05$) (Fig. 3). The number of predatory mites sampled on the various cultivars fluctuated between 0 and 6 mites per five leaves for banker plants and 0–3 mites per five leaves for direct-release treatments. In the banker plant treatment and the direct-release method, cultivars 992-7141 and FPP7039 held the highest seasonal mean numbers of *A. swirskii*, respectively (Fig. 3).

Discussion

Our study shows that *A. swirskii* could survive without prey on the most of screened pepper cultivars for at least 6 weeks in the presence of flowers, and 4 weeks when pre-planting-stage seedlings were provided with pollen. Amongst the 17 cultivars tested for the effect of pollen on mite establishment on pepper seedlings, a high mean density of predatory mites was recorded on 15 cultivars with pollen. This suggests that an initial pollen supply was important for mite survival and reproduction in the absence of their prey, flowers, or other plant-provisioned food sources. In a related study, Kutuk and Yigit (2011) reported that *A. swirskii* was able to feed and survive on pine pollen added to pepper seedlings for 2 weeks

Table 4 Mean number (±SEM) of *Amblyseius swirskii* per five top leaves of sweet pepper transplants on various sampling dates (weeks 1–8)

Pepper cultivars	1	2	3	4	5	6	7	8
7141	6.66 ± 0.66	10.0 ± 1.59ab	24.16 ± 2.90a	9.16 ± 1.90bc	39.16 ± 6.03ab	30.33 ± 7.51a	12.0 ± 1.82a	2.50 ± 0.42
992-7141	4.16 ± 1.13	18.16 ± 3.13a	15.66 ± 3.65abc	34.83 ± 5.54a	47.66 ± 7.01a	32.0 ± 8.20a	13.83 ± 1.07a	1.33 ± 0.55
FPP7039	5.55 ± 1.50	9.16 ± 1.01ab	14.0 ± 2.06abc	17.0 ± 4.91b	18.66 ± 3.14 cd	10.66 ± 3.92bc	3.50 ± 1.43b	0.66 ± 0.21
FPP9048	6.50 ± 0.61	7.50 ± 1.52ab	18.0 ± 3.94ab	15.16 ± 4.67bc	14.0 ± 2.23 cd	15.66 ± 3.54ab	3.0 ± 0.89b	1.0 ± 0.68
Bayonet	5.55 ± 0.56	5.50 ± 0.84b	4.16 ± 1.16d	5.0 ± 1.21c	27.33 ± 3.38abc	9.50 ± 1.05bc	16.83 ± 2.74a	0.16 ± 0.16
Cutlass	4.33 ± 0.91	7.83 ± 1.19ab	21.0 ± 4.83ab	15.33 ± 1.92b	9.66 ± 1.83d	4.83 ± 0.79bc	4.16 ± 3.18b	2.50 ± 0.34
Hunter	6.33 ± 0.84	6.50 ± 0.76ab	5.50 ± 1.05 cd	11.66 ± 6.29bc	19.50 ± 5.17 cd	4.33 ± 1.14c	1.33 ± 0.55b	2.16 ± 0.54
Tom Cat	4.50 ± 0.31	5.66 ± 0.80b	9.50 ± 1.20bc	10.33 ± 1.80bc	21.33 ± 1.80bcd	11.50 ± 2.39bc	0.66 ± 0.33b	3.16 ± 0.60

Means within a column followed by the same letter are not significantly different (Tukey's test $P > 0.05$)

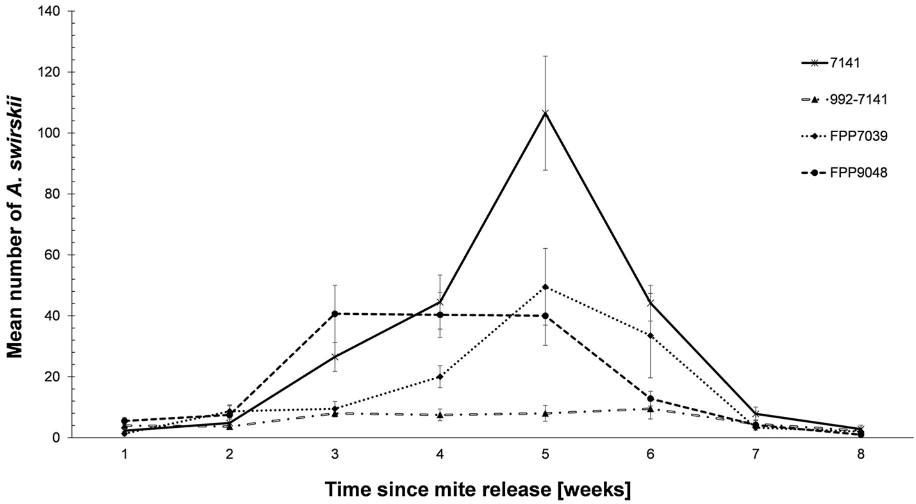

Fig. 2 The mean number (±SEM) of *Amblyseius swirskii* recorded weekly per five leaves of pepper transplants

in the absence of prey and flowers. Ramakers (1995) also showed successful establishment of *Iphiseius* (*Amblyseius*) *degenerans* (Berlese) on cucumber plants using a bee-collected pollen suspension in the absence of prey or any other host. In a concurrent study, we tested the PIF approach on seedlings of various pepper cultivars supplied with an artificial diet, and found that predatory mite populations established during the seedling stage of the hosts with a single release of mites. This indicates that early establishment of a generalist phytoseiid can be ensured on seedlings in the absence of prey if an alternative food source is provided. Thus, we suggest that PIF has potential to serve as an important tool not only for field growers but also for nursery growers. This approach will reduce their input cost for pest management on vegetable seedlings and increase the value of their product.

While screening pepper cultivars, low mite densities were observed initially (during pre-flowering stage), and their population increased after week 2 of the study. Depending on the initiation of flowering, predatory mite populations peaked between weeks 3 and 5, and reached moderate-low levels towards the end of this study, coinciding with the end of the flowering stage. These results suggest that flowers played an important role in sustaining predatory mite populations on pepper cultivars. Our results are consistent with several other studies that report a positive effect of pollen on various generalist phytoseiid mite species. Van Rijn et al. (2002), Ragusa et al. (2009), Nomikou et al. (2010), Kutuk and Yigit (2011) demonstrated that the supply of pollen as food resulted in a population increase of the phytoseiid mite species *I.* (*A.*) *degenerans*, *Cydnodromus californicus* McGregor and *A. swirskii*, and subsequently resulted in decreased pest populations on the host plants.

High densities of mites were observed in the experiment where 20 mites were released per plant, even reaching densities of >100 mites/plant. This suggests that host plants offered sufficient resources to support high mite densities, and that 20 mites/plant would be an effective release rate for greenhouse applications. We speculate that in addition to plant pollen, the presence of domatia and their emergence at different growth stages of pepper also affected mite densities in our study. During the screening of the pepper cultivars,

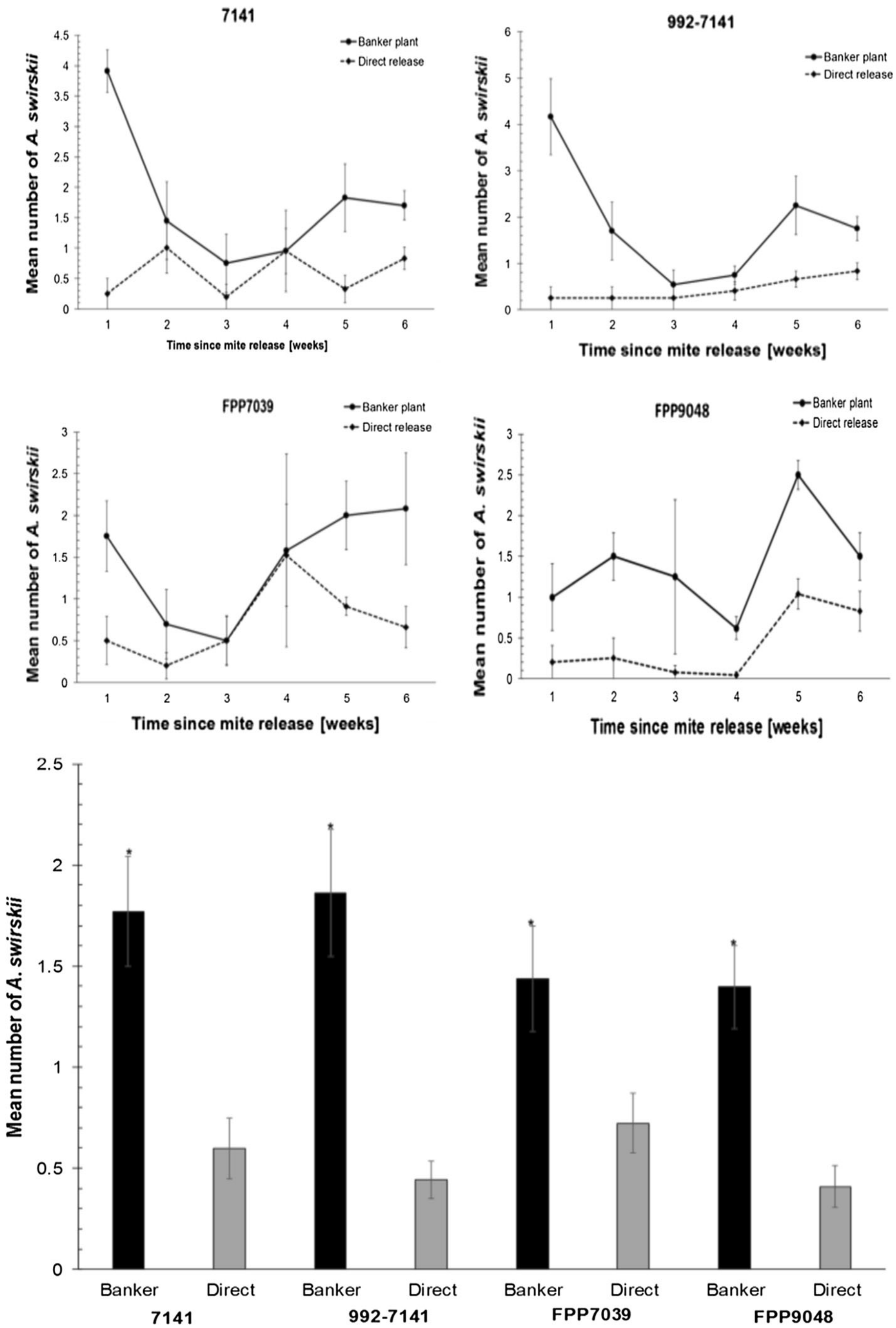

Fig. 3 The mean number (±SEM) of *Amblyseius swirskii* recorded weekly per five leaves in banker and direct-release treatments. Figure at the *bottom* shows seasonal means of *A. swirskii* recovered in two treatments. *Asterisks* indicate significant differences in *A. swirskii* abundance on a pepper cultivar between banker plant versus direct-release treatments

domatia appeared in week 2 after transplanting, along with the onset of flowering, coinciding with an increase of densities of *A. swirskii*. Although quantitative data on the number of domatia per pepper cultivar were not collected, weekly surveys on the presence or absence of domatia suggest that there was an apparent influence of its presence on the predatory mite populations. In the past, the role of domatia has been positively correlated with mite abundance by Romero and Benson (2005) and Loughner et al. (2008). It has been reported that domatia can help mite populations to increase by providing refugia for development and breeding; protecting against insecticides, natural enemies, intra-guild predation and adverse climatic condition; reducing the chance of dislodging from the host plant surface, as well as capturing plant pollen or fungal spores which might serve as sources of nutrition (Walter and O'Dowd 1992; Grostal and O'Dowd 1994; Walter 1996; Roda et al. 2000; Faraji et al. 2002; Ferreira et al. 2008, 2011; Avery et al. 2014). All these studies suggest that plant phenology plays an important role in supporting mite populations, and PIF can utilize these plant characteristics to establish mites during pre- and post-transplant stages of the crop prior to pest arrival. Nevertheless, more studies are needed to determine the potential role of domatia on the growth of mites in our system.

Our study also demonstrates that banker plants were a more effective mode to disperse *A. swirskii* to pepper transplants than direct releases of predators on the plants. The idea of using banker plants is related to conservation biological control, which provides ecological infrastructures required to sustain a reproducing population of natural enemies (Osborne and Barrett 2005; Frank 2010; Huang et al. 2011). We have demonstrated the role of banker plants in the establishment of biological control agents on host plants as well as in suppressing multiple pest populations previously (Xiao et al. 2011a, b, 2012; Avery et al. 2014). The results of our current study confirm those of our earlier studies, but they are novel in providing a comparison of the two methods. In the current study, the number of *A. swirskii* sampled every week in the screening experiments was several times higher than observed in the direct-release treatment in the banker versus direct-release experiment. An important difference between these experiments was that transplants in the direct-release treatment lacked an initial provisioning of pollens, unlike the pepper cultivar screening experiments. Thus, we suggest that the lack of food (pollen) at the time of mite release negatively affected mite establishment on the pepper transplants, resulting in a low recovery during the entire study period.

Conclusion

Various studies suggest that the initial application of pollen and host plant characteristics are important factors for predator establishment and should be taken into consideration before testing the PIF approach in commercial production. In the early growth stage of pepper (seedling or early transplant), pollen acts as a nutritional supplement and helps predatory mites to establish in the absence of their prey and, once established, mites can control the pest population in its incipient stage. Based on a series of screening tests, four pepper cultivars were found to warrant further testing. In future studies, two of these four varieties will be used to test the applicability of the PIF approach in protected and open pepper production units during various cropping seasons. The two release methods (direct vs. indirect) will also be evaluated under field conditions. If successful, this pest management method will increase the reliability of biological control strategies and reduce overall insecticide use.

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