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"Predator-In-First": A Preemptive Biological Control Strategy for Sustainable Management of Pepper Pests in Florida

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Abstract: The early establishment of a biocontrol agent in the production system, whether in the greenhouse, nursery, or field, is essential for the success of the biological control program, ensuring growers' profitability. In an effort to develop a sustainable pest management solution for vegetable growers in Florida, we explored the application of a preemptive biological control strategy, "Predator-In-First" (PIF), in regulating multiple pepper pests, *Bemisia tabaci* Gennadius, *Frankliniella occidentalis* Pergande, and *Polyphagotarsonemus latus* Banks under greenhouse and field conditions during different growing seasons. In these studies, two bell pepper cultivars (7039 and 7141) and the phytoseiid mite *Amblyseius swirskii* Athias–Henriot were used as a model system. Pepper seedlings (~8 week) of each cultivar were infested with varying rates of *A. swirskii* (20 or 40 mites/plant or one sachet/10 plant) and allowed to settle on plant hosts for a week before planting in pots or field beds. Results showed a comparative consistent performance of the treatment with the high rate of phytoseiids (40 mites/plant) in regulating *B. tabaci* and *F. occidentalis* populations in greenhouse studies, and *B. tabaci* and *P. latus* pests under field conditions. During two fall field seasons, higher marketable yields of 12.8% and 20.1% in cultivar 7039, and 24.3% and 39.5% in cultivar 7141 were observed in the treatment with the high rate of phytoseiids compared to the untreated control, indicating yield benefits of the approach. The outcome of the study is encouraging and demonstrates that PIF can be an important tool for organic vegetable growers and a potential alternative to chemical-based conventional pest management strategies. The advantages and limitations of the PIF approach in Florida pepper production are discussed.

Keywords: *Amblyseius swirskii*; *Bemisia tabaci*; *Frankliniella occidentalis*; IPM; horticulture; phytoseiid

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1. Introduction

Within the United States, the southeastern region has certain climatic advantages such as an extended winter photoperiod, mild temperature, and high light intensities conducive for optimal growth of a wide range of crops throughout the year [1]. In Florida, these parameters offer growers a strong foundation for the development of a multi-billion-dollar horticultural industry, ranked second in the country after California [2]. However, favorable climatic conditions, along with diverse flora present in this coastal state with 2170 km of shoreline and over 20 port-of-entries for plant products, also favor the introduction and establishment of a long list of invasive pests. Based on the Florida Department of Agriculture and Consumer Services (FDACS) records, between 1986 and 2000, >100 species of invasive arthropods established in Florida at the rate of ~1 species/month [3]. The continuous influx of invasive pests in the state, with growing concerns of using certain chemical classes, is an important driver pushing the need for alternate control strategies for both conventional and organic growers.

For the success of a sustainable biological control program, the establishment of the selected agents in the production system (greenhouse, nursery, field) is of paramount importance. In the past, there have been multiple strategies proposed for the long-term establishment of beneficials in the production system. Some of these include "Open Rearing" (also known as Banker Plants) systems [4–6], "Pest-In-First" strategies [7–9], "Slow Release" systems [10], and the use of artificial diets attracting natural enemies [11]. Although these strategies are known for their role in the conservation of beneficial agents, in turn, suppressing target pests, each of these has certain limitations impacting their broader acceptability. These may include the screening of non-crop (non-host pests) banker plant varieties; the release of non-pest arthropod species in the production system; dispersal of biological agents from sachets; and finding the chemicals selectively supporting the beneficial populations [12]. Considering the limitations of the above biological control strategies and needs of the Florida vegetable industry, we explored the utility of a relatively less known biological control concept, "Predator-In-First" (PIF), proposed by Ramakers [13]. The PIF approach combines aspects of inundative and conservational control strategies and aims to establish biological agents on the hosts during the seedling stage or early post-transplanting period before the arrival of any pests. The underlying theory behind the PIF approach is based on the characteristics of generalist predators' ability to survive on plant provisioned food (pollen, nectar) and morphological host plant characteristics, such as domatia that provide refugia for breeding and development, that allow establishment in the agroecosystem before the initial infestation of prey/pest populations. This is significant because natural enemies generally do not establish until later in the season when thrips or other pest numbers have built up [14–23].

In our studies, pepper plants were used as a model crop system, and the key component of this method involved the release of specific predatory mites *Amblyseius swirskii* Athias–Henriot on un-infested seedlings before transplanting for commercial production in the greenhouse or the field. The selection of the plant host and the predator species was influenced by our years of experience in assessing multi-trophic interactions with the ornamental banker plant system and the pest spectrum associated with commercial pepper production in the region, including *Bemisia tabaci* Gennadius, *Frankliniella occidentalis* Pergande, and *Polyphagotarsonemus latus* Banks [12,19,24–26]. In addition to causing direct damage to its hosts, these particular whitefly and thrips species hold the potential to vector plant damaging viruses and propensity to develop resistance against chemical insecticides. Thus, in order to meet the demand for vegetable production and related jobs, it is essential to simplify growers' concern by delivering new management strategies.

In the past, Kutuk and Yigit [27] showed the importance of pre-establishment of *A. swirskii* on pepper plants using *Pinus brutia* (Ten.) pollen in reducing F. occidentalis populations under greenhouse conditions. However, apart from a few greenhouse studies [13,27], the PIF approach has never been tested extensively under field conditions with multi-pest infestations. In the first part of the project [26], we focused on screening commercially grown pepper cultivars (hot and sweet) for their ability to sustain *A. swirskii* populations (with or without pollen), and assessed methods for mass inoculating seedlings with *A. swirskii* before planting in the greenhouse. Based on these studies, two out of 29 pepper cultivars were selected for further testing. Thus, in the current study, we evaluated the application of the PIF approach in managing multiple pests in bell peppers under commercial grower production standards. The specific objectives of this research were to assess the optimal rate (0, 20, 40 mites per plant) and overall performance of phytoseiid mites against multiple pests using the PIF approach with the top two pepper cultivars under two different environments (greenhouse and field) and growing seasons (fall and spring) in Florida.

2. Materials and Methods

A series of research studies were conducted in a protected greenhouse and outdoor field conditions at the United States Horticulture Research Laboratory (USHRL), USDA-ARS, Fort Pierce, FL (27.43 N, 80.40 W). The climatic conditions under the greenhouse were affected by outdoor temperature and relative humidity. *Amblyseius swirskii* populations used in the study were obtained from Koppert Biological Systems, USA (Howell, MI). Upon arrival, mites were stored at the most for 24 h in a growth chamber maintained at 12 ± 2 °C, RH 60 ± 5%, and 14:10 h L:D until the day of release. Two commercial bell pepper cultivars—7039 (Sakata Seed America Inc., Salinas, CA, USA) and 7141 (Seminis Vegetable Seeds, Inc., St. Louis, MO, USA)—were selected based on the results obtained in previous screening studies [26]. These pepper cultivars showed promise in supporting the survival of phytoseiid mites in the absence of any prey for a considerable amount of time.

2.1. Greenhouse Studies

Seeds of two bell pepper cultivars were grown in Premier Pro-Mix General Purpose Growing Medium in three separate transplant trays (Speedling^{TM}, Ruskin, FL, USA) (total 6 trays). Speedling^{TM} trays were placed inside plexiglass screened cages (61 cm × 91 cm × 61 cm) for ~8 weeks to prevent insect contamination before experimentation. Plants selected for the study were healthy, young, vigorous, and free of arthropod pests. Eight-week-old seedlings of each pepper cultivar (7039 and 7141) were inoculated with three different rates of *A. swirskii*: (1) 20 *A. swirskii*/plant, (2) 40 *A. swirskii*/plant, and (3) untreated control—without *A. swirskii*, making a total of six treatments. *Amblyseius swirskii* counts in bran (carrier material) were standardized and released on pepper seedlings following the protocol of Kakkar et al. [28]. The bran volume was quantified to determine the desired number of predatory mites (20 and 40/plant) by repeated drawings from the bottle in which the product was received from the company. The carrier material ensured initial support to the phytoseiid mites needed to settle on the host. They were allowed to establish on pepper seedlings for seven days before transplanting into 3.78 L plastic pots. The experiment was conducted in a randomized complete block design with six replicates, and each plot consisted of three plants (18 plants per treatment). For each treatment, potted seedlings were placed in a large saucer filled with water, and the plants did not touch each other. Plants were irrigated as needed (~3 times a week) and fertilized with 800 mL/pot of Peters Professional® 20-10-20 (325 ppm) (Scotts Co., Marysville, OH, USA) once a week.

For the efficacy evaluation of the PIF approach, pepper plants were moved to a greenhouse harboring natural populations of *B. tabaci* (Middle Eastern Asia Minor 1, MEAM1) and *F. occidentalis*. Developmental stages of arthropods including *B. tabaci* (eggs and nymphs), *F. occidentalis* (larvae), and *A. swirskii* (eggs and motile) were recorded weekly on five randomly selected leaves from the top of each pepper plant by direct count sampling method using a 30× handheld lens for a period of eight week. Once plants reached flowering stage, three flowers/plant were collected in 50 mL flat top Falcon tubes (USA Scientific, Ocala, FL, USA) and brought to the USHRL laboratory. Foliage samples were rinsed in 20 mL of 70% ethanol for 30 min to dislodge *F. occidentalis* larvae and *A. swirskii* motile. The contents in the tube were separated using a 25-µm grading sieve (USA Standard Testing Sieve, W. S. Tyler, Inc., Mentor, OH, USA). Mites trapped inside the sieve were flushed with 75% ethanol into 6 cm Petri dishes (USA Scientific) with artificial grids, and the number of various life stages was counted under a stereomicroscope at 12.5× (Olympus SZX12).

2.2. Field Studies

Field evaluations of the PIF approach were conducted over a two-year period during three consecutive pepper growing seasons (fall, spring) in Florida. The two main objectives of the field trials were to optimize the rate of phytoseiid mites released per plant and determine the best mode of mite application: (1) direct application—mites released directly on the seedlings as in the greenhouse assay or (2) indirect application—mites released using commercial slow-release sachet (Swirski-Mite LD, Koppert Biological Systems). The slow-release sachets can provide protection and food to predators and improve their survival in unfavorable environmental conditions. Seeds of individual bell pepper cultivars were sown in 128 cell Speedling^{TM} transplant trays following a similar protocol mentioned above. The method of plant propagation, maintenance, and mite treatment was similar to the greenhouse trial except that four different rates of mites were used and two methods of application making a total of eight treatments. Rates were (1) 20 *A. swirskii*/plant, (2) 40 *A. swirskii*/plant, (3) 1 mite sachet/10 plants, and (4) untreated control—without *A. swirskii*.

Field Preparation and Treatment Evaluations

Studies were conducted at the USHRL experimental farm site where six raised beds (150 m long, 0.91 m wide, 0.15 m high, and 1.5 m spacing between centers) of Oldsmar sand (sandy, siliceous, hyperthermic Alfic Arenic Alaquod) were prepared. An alternative method to methyl bromide fumigation–anaerobic soil disinfestation combined with soil solarization was adopted to control weeds and soil-borne pathogens, as well as plant-parasitic nematodes following the protocol of Butler et al. [29]. Each of the six beds was divided into eight 12 m long treatment plots with double rows of pepper planting. A 6.09 m long buffer zone was maintained between two adjacent treatment plots. All eight treatments (two cultivars and four mite rates) were arranged in a randomized complete block design with six replications. Irrigation, fertilization, weed, and disease management were achieved following standard cultural practices [30]. In order to control caterpillar damage (*Spodoptera* spp., *Helicoverpa* spp.), *Bacillus thuringiensis*-based insecticides Xentari DF® (var. kustaki) at 1.2 L/ha (Valent Biosciences Corporation, Libertyville, IL, USA) and DiPel DF® (var. kurstaki) at 1.1 kg/ha [31] were used. No other chemical insecticides were used in these studies.

Sampling was conducted weekly from each plot for a period of eight weeks during two fall studies and 10 weeks in the spring study. The sampling unit consisted of randomly selecting 10 top leaves per plot, collected in labeled plastic Ziploc® bags (17 × 22 cm). Once flowering was initiated, 10 flowers/plot were collected every week in 50 mL Falcon conical tubes. Foliage samples were brought to the USHRL laboratory and processed following the protocol mentioned above. Leaf and processed flower samples were observed for various life stages of *B. tabaci* (eggs and nymphs), *F. occidentalis* (larvae), *P. latus* (eggs and motile), and *A. swirskii* (eggs and motile) using a stereomicroscope at 12.5× (Olympus SZX12).

During the first fall season of field trials, we received >30 cm of rain, beginning just days after transplanting. Upon completion of the first three samplings, an augmentative release of mites was done based on the low retrieval of *A. swirskii* in the treatment plots due to very heavy storms coupled with strong winds for prolonged periods negatively impacting mites and especially sachets. Normal seasonal rain was experienced in the second and third trials, but the second augmentation was repeated for trial comparisons. In the plots receiving *A. swirskii* through the direct release method, the same number of *A. swirskii* were released on the plants at the beginning of the study. Two treatments with an indirect release through sachets were supplemented by 1 banker plant/10 field plants. Banker plants were made by using the same aged group pepper seedlings (kept in arthropod-free environment) transplanted in 3.78 L plastic pots and inoculated with 100 mites/plant. Mites were allowed to establish on the host plant for seven days before adding in the treatment plots. Treatments with 7039 and 7141 peppers received banker plants from respective pepper varieties. The decision to replace mite sachets with banker plants as an indirect release method was influenced by (1) the observation on the impact of wind and rain on mite sachets and (2) efficiency of pepper banker plants as a reservoir and medium of mites release reported in our associated studies [19,20,25,26].

Challenges from additional arthropod pests were encountered during the three growing seasons of commercial bell pepper field production that we evaluated. Apart from two target pests (*B. tabaci* and *F. occidentalis*), there was a heavy infestation of *P. latus* observed during both fall growing seasons. Since *A. swirskii* is a generalist predator, the fortuitous incidence of *P. latus* benefited the scope of the trial by adding another pest to the spectrum when evaluating the utility of the PIF approach. During the spring season, an extremely high pest pressure of pepper weevil, *Anthonomus eugenii* Cano was observed, which in the absence of appropriate chemical remediation measures severely impacted pepper yield (Supplementary Figure S1). Consequently, yield data is presented only for the two fall pepper growing seasons. Extrapolated yield per treatment (kg/ha) for two fall seasons were calculated by converting yield obtained per treatment by using the formula below:

Extrapolated yield (kg/ha) = [Yield obtained per treatment (kg/acre) × 2.4 (conversion unit for acre to Kg/ha)]/(Total treatment size = ~0.033 acre per treatment)

2.3. Statistical Analysis

Data from greenhouse and field efficacy experiments were analyzed independently for each season using a generalized linear mixed model with the SAS® [32] procedure GLIMMIX. In respective experiments, a generalized linear mixed model was fit to the data to determine the effect of treatment, sampling period (time in weeks), and their interaction (treatment * week) on arthropods growth stage counts, separately. The sampling week was considered a repeated measure, and replicate was treated as a random effect in the linear mixed model. The first-order autoregressive correlation structure (*type* = *ar* (1)) was applied to account for the correlation in observations, generated by re-sampling the same experiment unit over time. The data were normalized using square root transformation to stabilize heterogenous variance before analysis. When the interaction of treatment and time was found to be significant, mean separations were run for differences in treatments in the same time period. In two out of the three field seasons, the effect of treatments was not observed on the *F. occidentalis* population recorded in flowers. For comparison, the seasonal mean of *F. occidentalis* and *A. swirskii* counts is presented in the supplementary figures. Pepper yield from the two fall field trials was analyzed using a one-way analysis of variance. Differences among treatment means were separated using Fisher's LSD test ($\alpha = 0.05$). The data presented are the untransformed means.

3. Results

For the sake of simplicity, results presented below include life stages of *B. tabaci* (eggs + nymphs), and *A. swirskii* (eggs + motile) observed on the leaves and *F. occidentalis* (larvae) and *A. swirskii* (motile) recorded in the flower samples over various sampling weeks.

3.1. Evaluation of the PIF Approach under Greenhouse Conditions

3.1.1. Fall Greenhouse Study

During the fall greenhouse study, both the main effects (treatment and time) had a significant effect on *B. tabaci* and *A. swirskii* populations recorded on the pepper leaves (Supplementary Table S1). The effect of treatments on the abundance of pests and the predator varied over time, explaining the treatment * week effects. The *B. tabaci* population was low at the beginning and increased rapidly after weeks 4–5. A significant suppression in the *B. tabaci* population was observed in all phytoseiid treatments compared to two untreated plots for all sampling dates between weeks 2 and 8. (Figure 1a). No significant differences in the activity of two phytoseiid rates (20 vs. 40) were observed except in weeks 5 and 7. The overall *B. tabaci* suppression (over 8 weeks) in the phytoseiid treatments of two pepper cultivars compared to untreated plots of the respective cultivars were 75.9% and 89.4% in treatments 7039_20 and 7039_40 (compared to 7039_0), and 70.5% and 80.3% in 7141_20, and 7141_40 (compared to 7141_0). The population of *A. swirskii* followed a similar trend to *B. tabaci*, and it was low during the initial week and peaked towards weeks 6–7 (Figure 1b). A significantly higher number of *A. swirskii* was reported in all phytoseiid treatments than in the two untreated plots on all the sampling dates between weeks 2 and 8. No significant differences in the abundance of *A. swirskii* in two phytoseiid treatments (20 vs. 40) were observed except in weeks 5 and 8.

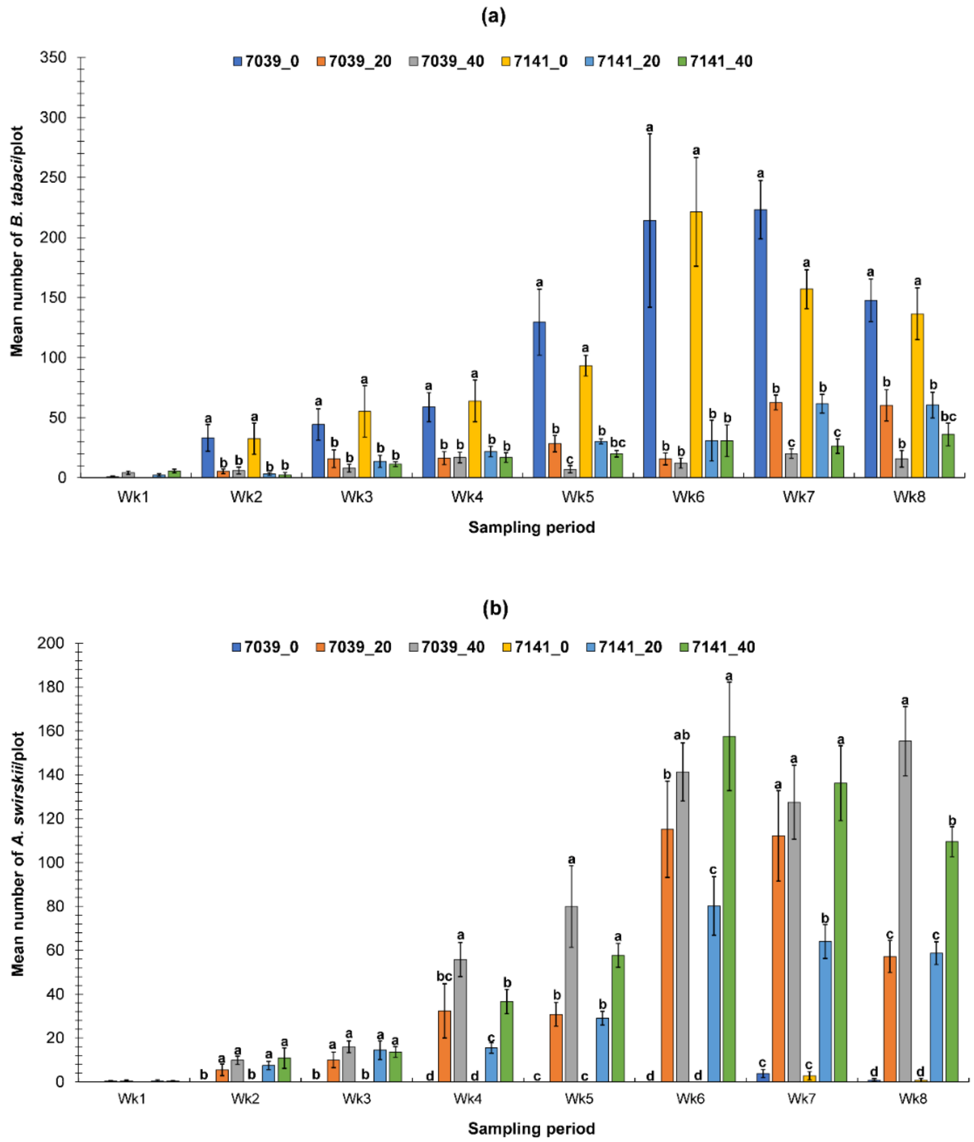


Figure 1. Mean number (±SE) of (**a**) *Bemisia tabaci* and (**b**) *Amblyseius swirskii* life stages observed per plot on pepper leaves in various sampling weeks during fall greenhouse study. The first part of the treatment name represents pepper cultivar, and the second part shows the number of *A. swirskii* received per seedling before transplanting in the pots. Treatment bars within a sampling period with different letters are significantly different (Fisher's LSD test, $p < 0.05$).

During the fall trial, flower samples were collected from weeks 2–8. There was a significant effect of treatment rate and time on *F. occidentalis* larvae and *A. swirskii* motile population recorded in the flower samples, whereas the effect of treatment * week interaction was observed only on *F. occidentalis* (Supplementary Table S1). Except for 7141_40, none of the phytoseiid treatments showed significant suppression in *F. occidentalis* counts compared to two untreated plots on two sampling dates (Figure 2a). However, overall, a grower acceptable reduction rate of >70% (7039_20 = 74.5%, 7039_40 = 81.4%, 7141_20 = 87.0, and 7141_40 = 95.7) in the thrips larval abundance was observed in the phytoseiid treatments compared to respective untreated plots. A significantly higher number of *A. swirskii* was reported in the treatment 7039_40 than two untreated plots on all the sampling dates except on weeks 5 and 7, whereas in 7141_40 such difference was observed in weeks 5, 6, and 8 (Figure 2b). No significant differences in the abundance of *A. swirskii* in two phytoseiid treatments (20 vs. 40) were observed. Overall, significantly higher *A. swirskii* counts were observed on treatments 7039_40, 7141_40, and 7039_20 than 7141_20 ($p < 0.05$).

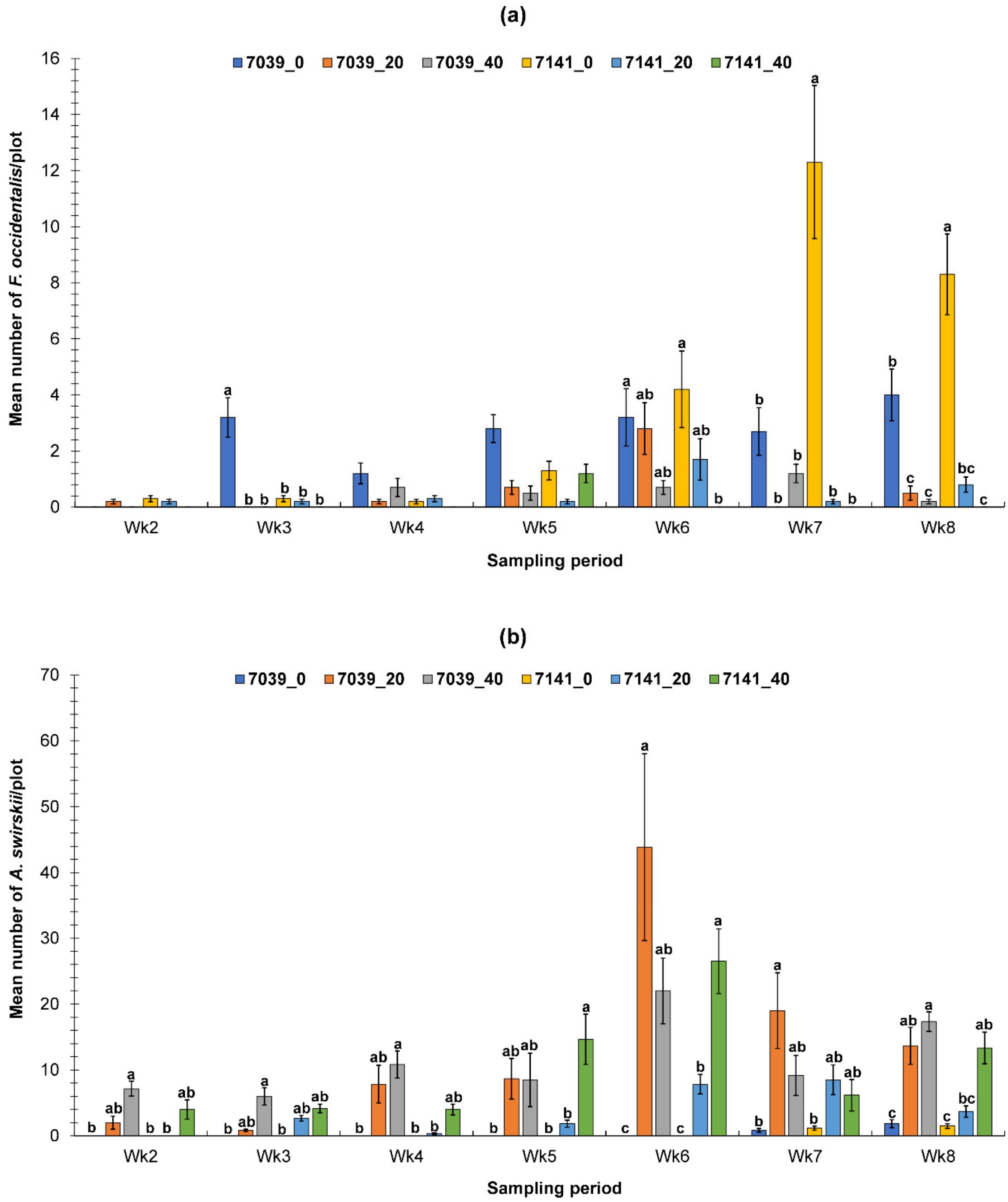


Figure 2. Mean number (±SE) of (**a**) *Frankliniella occidentalis larvae* and (**b**) *Amblyseius swirskii* motile observed per plot in pepper flowers on various sampling weeks during fall greenhouse study. The first part of the treatment name represents pepper cultivar, and the second part shows the number of *A. swirskii* received per seedling before transplanting in the pots. Treatment bars within a sampling period with different letters are significantly different (Fisher's LSD test, $p < 0.05$).

3.1.2. Spring Greenhouse Study

In the spring season, similar to the fall trial, there was a significant effect of treatment, time, and their interaction (treatment * time) on *B. tabaci* and *A. swirskii* populations (Supplementary Table S1) recorded on the host leaves. A significant suppression in the *B. tabaci* population was observed in all phytoseiid treatments compared to two untreated plots on the majority of sampling dates (weeks 3–8) (Figure 3a). No significant differences in the activity of two phytoseiid rates (20 vs. 40) were observed in any sampling period. The overall *B. tabaci* suppression (over 8 weeks) in the phytoseiid treatments of two pepper cultivars compared to untreated plots of the respective cultivars were 79.6% and 76.2% in treatments 7039_20 and 7039_40, and 70.6% and 73.8% in 7141_20 and 7141_40. During the spring season, a higher density of *A. swirskii* was reported on host leaves compared to the fall greenhouse study. The population of *A. swirskii* was low at the beginning of the study and increased rapidly and peaked during weeks 5–6 (Figure 3b). A significantly higher number of *A. swirskii* was reported in all phytoseiid treatments than two untreated plots on all the sampling dates between weeks 4 and 8. A significantly higher *A. swirskii* abundance was recorded on 7039_40 than the rest of the treated plots on weeks 5 and 6. No significant differences in the abundance of *A. swirskii* in two phytoseiid treatments (20 vs. 40) were observed.

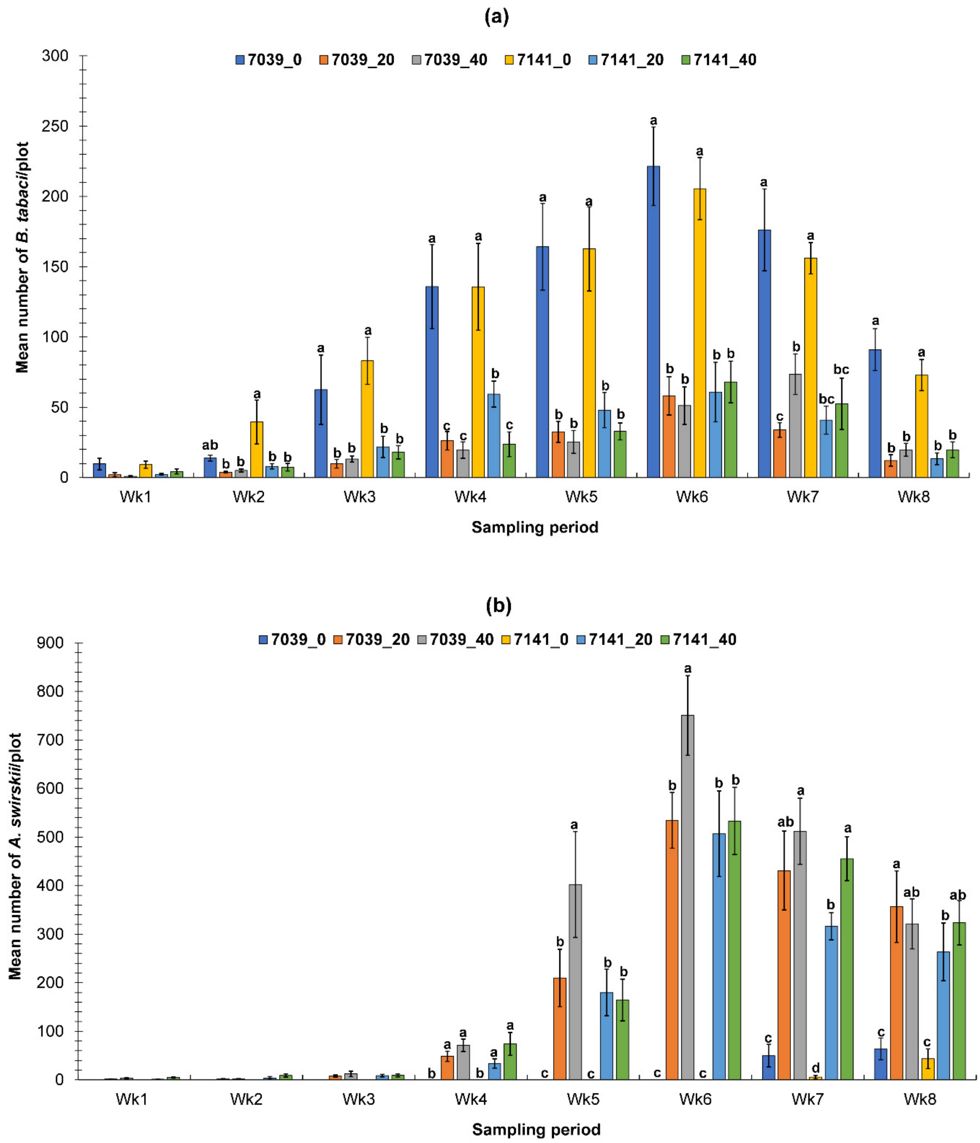


Figure 3. Mean number (±SE) of (**a**) *Bemisia tabaci* and (**b**) *Amblyseius swirskii* life stages observed per plot on pepper leaves in various sampling weeks during spring greenhouse study. The first part of the treatment name represents pepper cultivar, and the second part shows the number of *A. swirskii* received per seedling before transplanting in the pots. Treatment bars within a sampling period with different letters are significantly different (Fisher's LSD test, $p < 0.05$).

During the spring trial, there was a significant effect of treatment rate and time on *F. occidentalis* larvae and *A. swirskii* motile population recorded in the flower samples, whereas the effect of treatment * week interaction was not observed (Supplementary Table S1). No significant differences in *F. occidentalis* counts were observed in the phytoseiid treatments compared to two untreated plots on any of the sampling dates (Figure 4a). However, overall, a significant suppression in the thrips larval population was observed in the treatments 7039_40 and 7141_40 compared to two untreated plots. The seasonal thrips suppression in the phytoseiid treatments of two pepper cultivars compared to untreated plots were 35.4% and 65.9% in treatments 7039_20 and 7039_40, and 40.2% and 71.6% in 7141_20 and 7141_40. From the onset of flowering, a low-moderate count of *A. swirskii* was observed in the flower samples which peaked on week 6 aligning with the thrips population. Significantly higher numbers of *A. swirskii* were reported in the treatments 7039_40 and 7141_40 compared to the two untreated plots on weeks 6 and 7 (Figure 4b). However, no significant differences in *A. swirskii* counts were observed on phytoseiid treatments with two different rates (20 vs. 40) of application ($p > 0.05$).

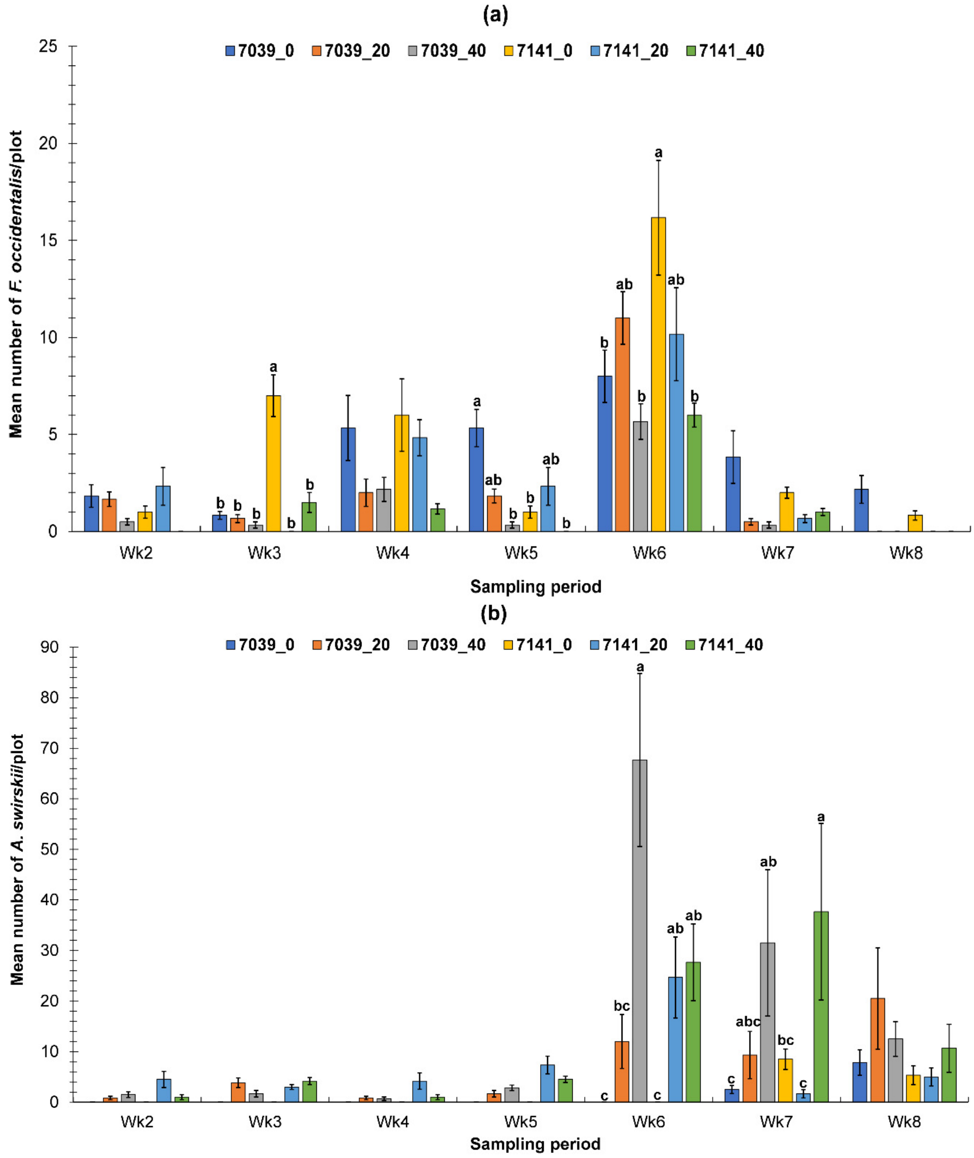


Figure 4. Mean number (±SE) of (**a**) *Frankliniella occidentalis* larvae and (**b**) *Amblyseius swirskii* motile observed per plot in pepper flowers on various sampling weeks during spring greenhouse study. The first part of the treatment name represents pepper cultivar, and the second part shows the number of *A. swirskii* received per seedling before transplanting in the pots. Treatment bars within a sampling period with different letters are significantly different (Fisher's LSD test, $p < 0.05$).

3.2. Evaluation of the PIF Approach Under Field Condition

3.2.1. Fall Field Study 1

In the first fall field season, both the main effects (treatment and time) and their interaction (treatment * time) had a significant effect on the pest *B. tabaci*, *P. latus*, and predator *A. swirskii* populations recorded on the pepper leaves (Supplementary Table S1). Weekly samplings showed overlapping generations of three arthropods on the host plants throughout the study period (Figure 5). The *B. tabaci* population was low at the beginning of the study and increased rapidly after week 3 and peaked during week 5, gradually decreased at week 7, and then maintained low-moderate levels towards the end. During weekly surveys, significantly lower counts of *B. tabaci* were observed on treatments 7039_40 and 7141_40 compared to two untreated plots on weeks 6–8 and two other lower phytoseiid direct application treatments (7039_20, and 7141_20) showed impact towards the end of the study on weeks 7–8. Sachet treatments (7039_sachet, 7141_sachet) were efficacious only in week 7 (Figure 5a). The overall *B. tabaci* suppression (over 8 weeks) in the phytoseiid treatments of two pepper cultivars compared to untreated plots of the respective cultivars were > 55% in the direct application method (7039_20 = 55.3%, 7039_40 = 59.2%, 7141_20 = 60.4%, and 7141_40 = 68.3%), and 42.1% (7039_sachet) and 45.5% (7141_sachet) for the sachet treatments.

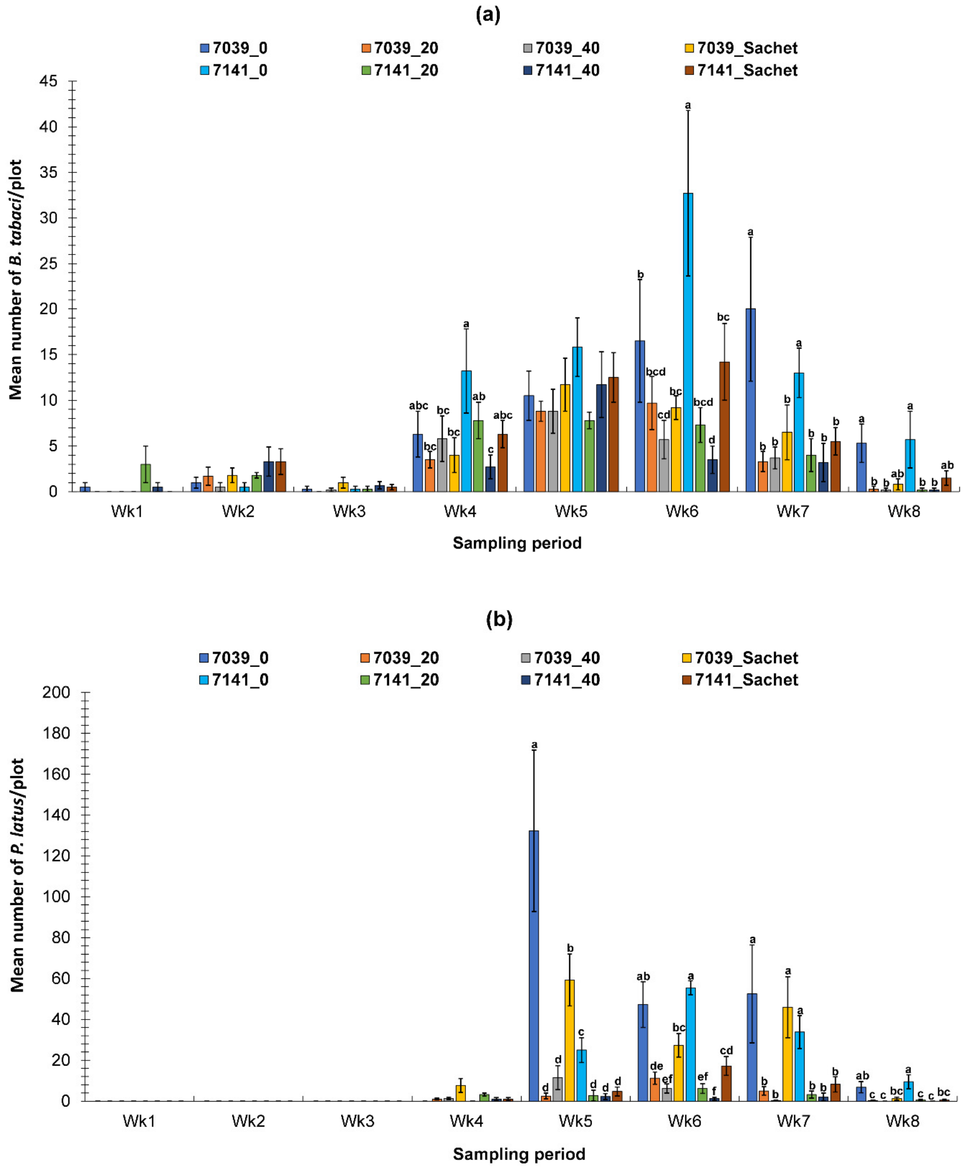


Figure 5. *Cont.*

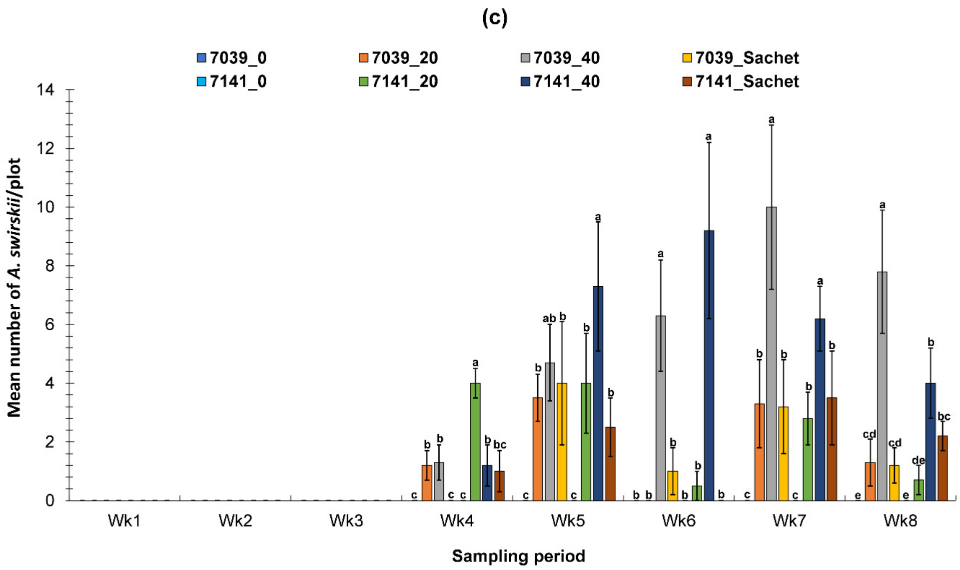


Figure 5. Mean number (±SE) of (**a**) *Bemisia tabaci*, (**b**) *Polyphagotarsonemus latus*, and (**c**) *Amblyseius swirskii* life stages recorded per plot on pepper leaves in various sampling weeks of the first field study (fall season). The first part of the treatment name represents pepper cultivar, and the second part shows the number of *A. swirskii* received per seedling before transplanting in the field. An augmentative release of *A. swirskii* was done after week 3. Treatment bars within a sampling period with different letters are significantly different (Fisher's LSD test, $p < 0.05$).

The *P. latus* population was not recorded during initial sampling, and its outbreak occurred during the middle of the study period (weeks 4–5) (Figure 5b). All treatments that received direct phytoseiid application (7039_20, 7039_40, 7141_20, and 7141_40) were efficacious on the majority of sampling dates (weeks 5–8) maintaining a low-moderate pest population throughout the study period. However, sachet treatments were not consistent in regulating the *P. latus* population. The overall *P. latus* suppression (over 8 weeks) in the phytoseiid treatments of two pepper cultivars compared to untreated plots were > 85% in the direct application method (7039_20 = 91.6%, 7039_40 = 92.0%, 7141_20 = 87.1%, and 7141_40 = 94.8%). In two sachet treatments, *P. latus* suppression was comparatively low, 40.8% (7039_sachet) and 74.8% (7141_sachet).

During initial sampling weeks, no *A. swirskii* life stages were recovered on the host leaves, so an augmentative release of phytoseiid was made after week 3 (Figure 5c). Following the population trend of *P. latus*, significantly higher numbers of *A. swirskii* were reported in phytoseiid treatments 7039_40 and 7141_40 than two untreated plots between weeks 4 and 8, whereas it was different from other treated plots on weeks 6–7. Overall, significantly higher *A. swirskii* counts were observed on treatments 7039_40 and 7141_40 than other phytoseiid treatments ($p < 0.05$).

During the first fall trial, flower samples were collected from weeks 3–8. There was a significant effect of time on the *F. occidentalis* population, whereas, *A. swirskii* abundance was affected with both the main effect and their interaction (Supplementary Table S1). No significant difference in the thrips population was recorded in any phytoseiid treatment compared to the two untreated plots (Supplementary Figure S2). A significantly higher number of thrips was observed on week 4 compared to the rest of the sampling period. The overall thrips suppression in the phytoseiid treatments of two pepper cultivars compared to untreated plots of respective cultivars were low: 17.1%, 39.9%, and 16.2% in treatments 7039_20, 7039_40, and 7039_sachet, and 40.4%, 54%, and 33.2% in 7141_20, 7141_40, and 7141_sachet. A significantly higher count of *A. swirskii* was observed in the treatments that received direct application of phytoseiid compared to two untreated plots (Supplementary Figure S2). Among phytoseiid treatments, 7141_40 exhibited higher *A. swirskii* than the two sachet treatments.

3.2.2. Spring Field Study

During the spring field season, the arthropod population was comparatively lower on the host leaves than the fall season. There was a significant effect of treatment, sampling period, and their interaction (treatment * week) observed on the *B. tabaci* and *A. swirskii* densities (Supplementary Table S1). During weekly surveys, significantly lower counts of *B. tabaci* were observed on all treatments (except 7039_20) that received direct phytoseiid application on weeks 6 and 7, whereas sachet treatments were effective only on week 7 (Figure 6a). The overall *B. tabaci* suppression in the phytoseiid treatments of two pepper cultivars compared to untreated plots were relatively higher (>85%) in the treatments 7039_40 and 7141_40 than rest of the treatments (7039_20 = 75.2%, 7039_40 = 87.6%, 7039_sachet = 79.3%, 7141_20 = 39.4%, 7141_40 = 85.9%, and 7141_sachet = 69.1%).

Unlike in the first field assay, the *A. swirskii* population was recovered during the initial sampling week which was low at the beginning and peaked during week 6, then maintained a low population towards the end of the study (Figure 6b). Treatment 7039_40 exhibited a significantly higher *A. swirskii* population compared to the two untreated plots in weeks 4-7, whereas 7141_20 and 7141_sachet showed promise on weeks 4–6 and 4, 6, and 7, respectively. Among treated plots, significantly higher numbers of *A. swirskii* were recorded (over 8 week period) on 7039_40 than 7039-20, 7039_sachet, and 7141_40 treatments.

The spring field planting season was long and flower samples were collected from weeks 4–10. There was a significant effect of treatment, time, and their interaction on *F. occidentalis* incidence, whereas the *A. swirskii* population was affected by sampling time (Supplementary Table S1). Except for treatment 7039_40, none of the phytoseiid treatments were efficacious in regulating the thrips population in the flowers (Supplementary Figure S3). The overall thrips suppression in the phytoseiid treatments of two pepper cultivars compared to the untreated plots of respective cultivars were lower than the previous season: 16%, 37%, and 0% in treatments 7039_20, 7039_40, and 7039_sachet, and 2%, 7%, and 0% in 7141_20, 7141_40, and 7141_sachet. In the treated plots, *A. swirskii* numbers were low throughout the study period, and no significant differences in *A. swirskii* numbers in phytoseiid treatments were observed compared to untreated plots.

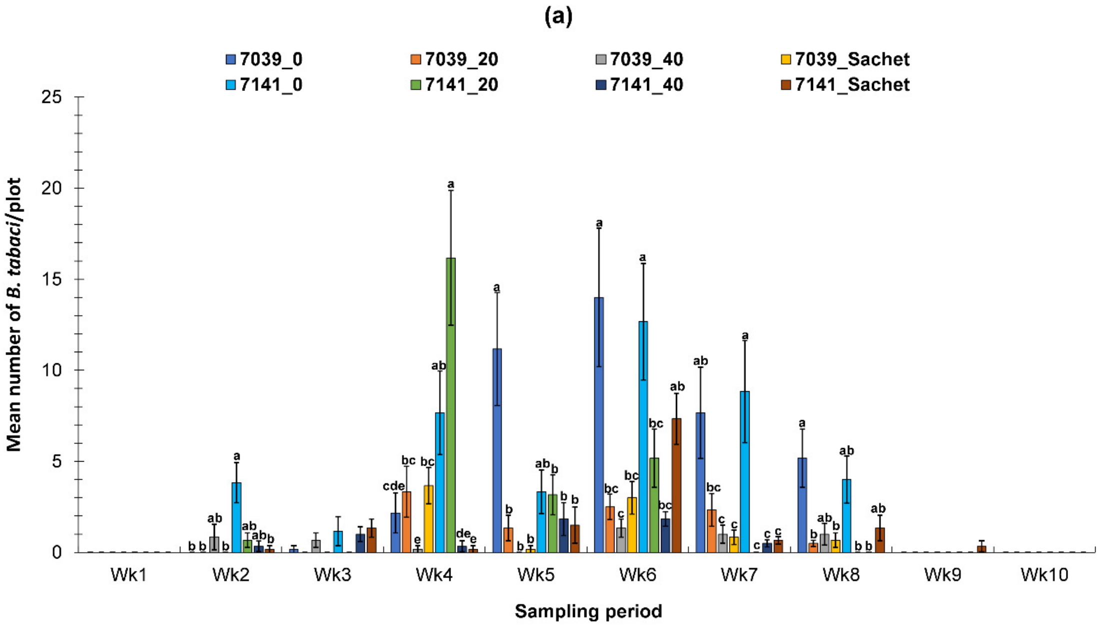


Figure 6. *Cont.*

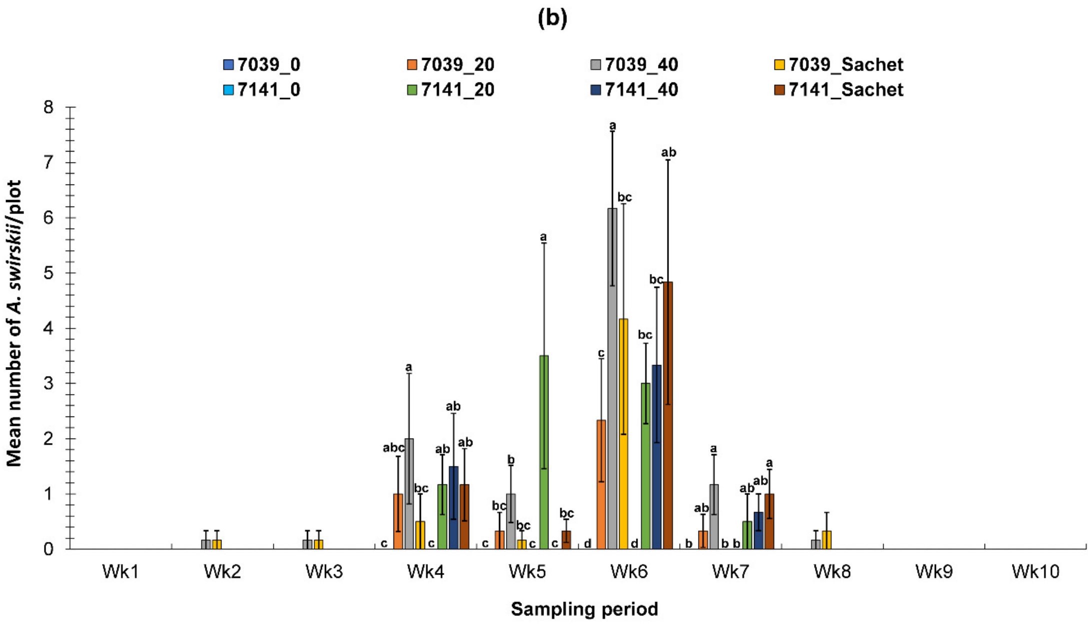


Figure 6. Mean number (±SE) of (**a**) *Bemisia tabaci* and (**b**) *Amblyseius swirskii* life stages recorded per plot on pepper leaves in various sampling weeks of the second field study (spring season). The first part of the treatment name represents pepper cultivar, and the second part shows the number of *A. swirskii* received per seedling before transplanting in the field. An augmentative release of *A. swirskii* was done after week 3. Treatment bars within a sampling period with different letters are significantly different (Fisher's LSD test, $p < 0.05$).

3.2.3. Fall Field Study 2

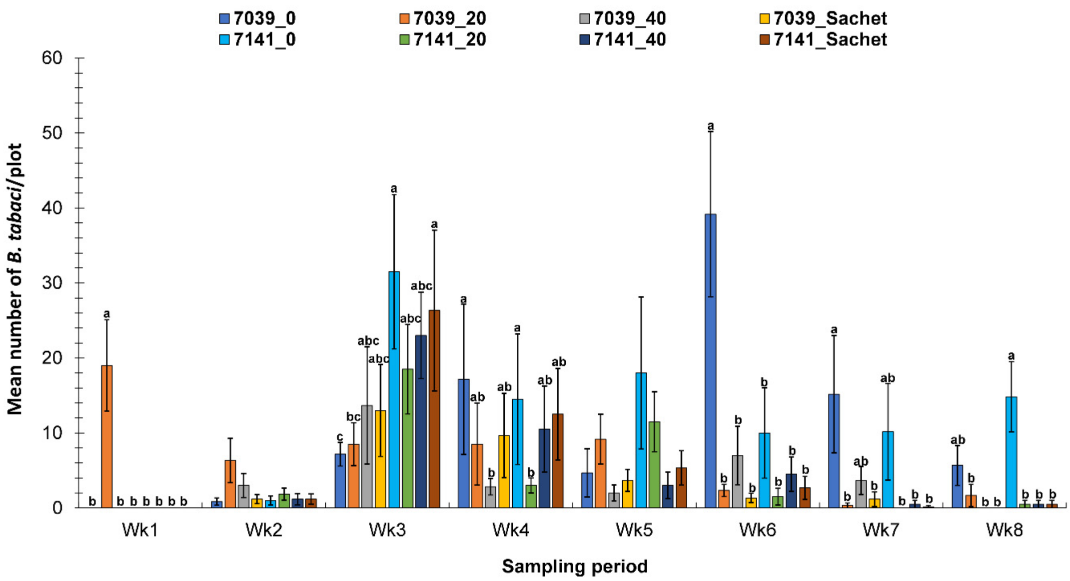
During the third field season, both the main effects (treatment and time) and their interaction (treatment * time) had a significant effect on the three arthropod populations recorded on the host leaves (Supplementary Table S1). None of the phytoseiid treatments were consistent in regulating the *B. tabaci* population during various sampling weeks (Figure 7a). The overall *B. tabaci* suppression (over 8 weeks) in the phytoseiid treatments were comparatively lower than the spring season (37.8%, 64.2%, and 66.6% in treatments 7039_20, 7039_40, and 7039_sachet, and 63.2%, 56.8%, and 51.3% in 7141_20, 7141_40, and 7141_sachet), but followed the similar trend as in the first fall season in the presence of *P. latus*.

The *P. latus* population started appearing on week 3 and peaked towards the middle of the study period (weeks 4–5) (Figure 7b). All phytoseiid treatments showed a significant effect on the *P. latus* population on weeks 5 and 6, where no differences among treatments with direct phytoseiid applications were observed. The overall *P. latus* suppression (over 8 weeks) in the phytoseiid treatments compared to untreated plots was >70% in the direct application method with maximum reduction observed in the treatment 7039_40 (7039_20 = 73.5%, 7039_40 = 85.4%, 7141_20 = 82.4%, and 7141_40 = 74.7%). In two sachet treatments, *P. latus* suppression was 74.7% (7039_sachet) and 63.7% (7141_sachet).

Unlike the previous fall season, the *A. swirskii* population was recovered during initial sampling weeks which peaked on weeks 5–6. Although all the phytoseiid treatments sustained low-moderate counts of *A. swirskii* throughout the study period, significantly higher numbers of *A. swirskii* (than two untreated plots) were reported in treatments 7039_40 on weeks 3–8, and weeks 3–7 in 7141_40 (Figure 7c). Overall, significantly higher *A. swirskii* counts were observed on treatments 7039_40 and 7141_40 than other phytoseiid treatments ($p < 0.05$).

During the second fall field study, flower samples were collected from weeks 3 to 8, and comparatively higher thrips abundance was recorded than the first season. There was a significant effect of treatment and time on the *A. swirskii* population, whereas *F. occidentalis* abundance was only affected by sampling time. No significant effect of treatment * week interaction was observed on any of the arthropod recovered in flowers (Supplementary Table S1). No significant difference in the thrips population was recorded in any phytoseiid treatments compared to the two untreated plots (Supplementary Figure S4). A significantly higher number of thrips was observed on week 3 than rest of the sampling period. The overall thrips suppression in the phytoseiid treatments of two pepper cultivars compared to untreated plots of respective cultivars were lower than the first fall season: 0%, 27%, and 0% in treatments 7039_20, 7039_40, and 7039_sachet, and 20%, 36%, and 6.7% in 7141_20, 7141_40, and 7141_sachet. A significantly higher count of *A. swirskii* was observed in the treatments 7039_40 and 7141_40 compared to two untreated plots (Supplementary Figure S4). Among phytoseiid treatments, no significant difference in *A. swirskii* abundance was observed. Significantly higher numbers of *A. swirskii* were recorded on weeks 4 and 5 than 6–8.

(a)



(b)

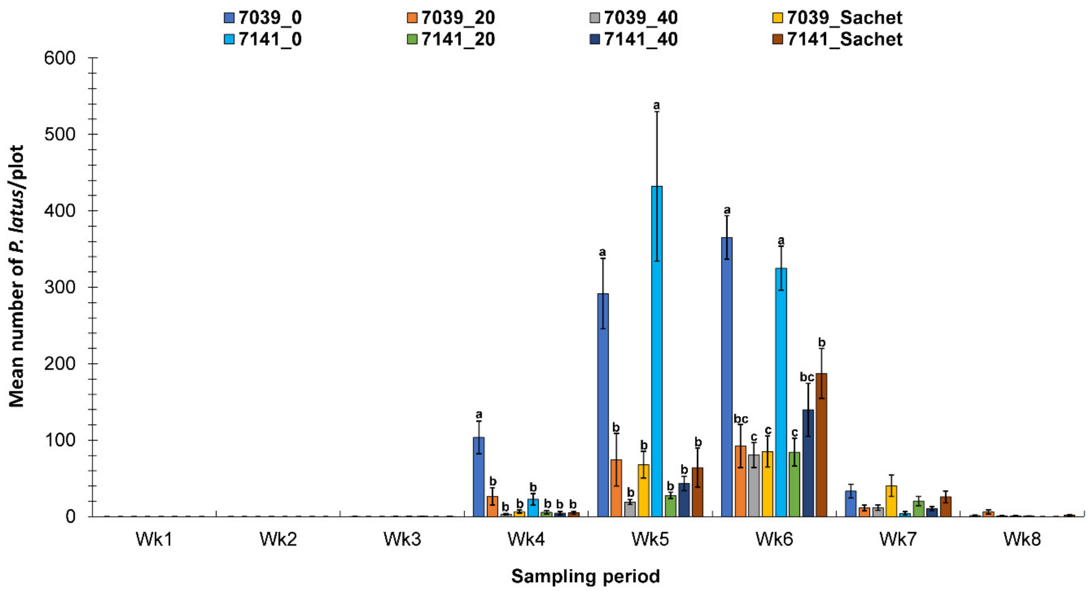


Figure 7. *Cont.*

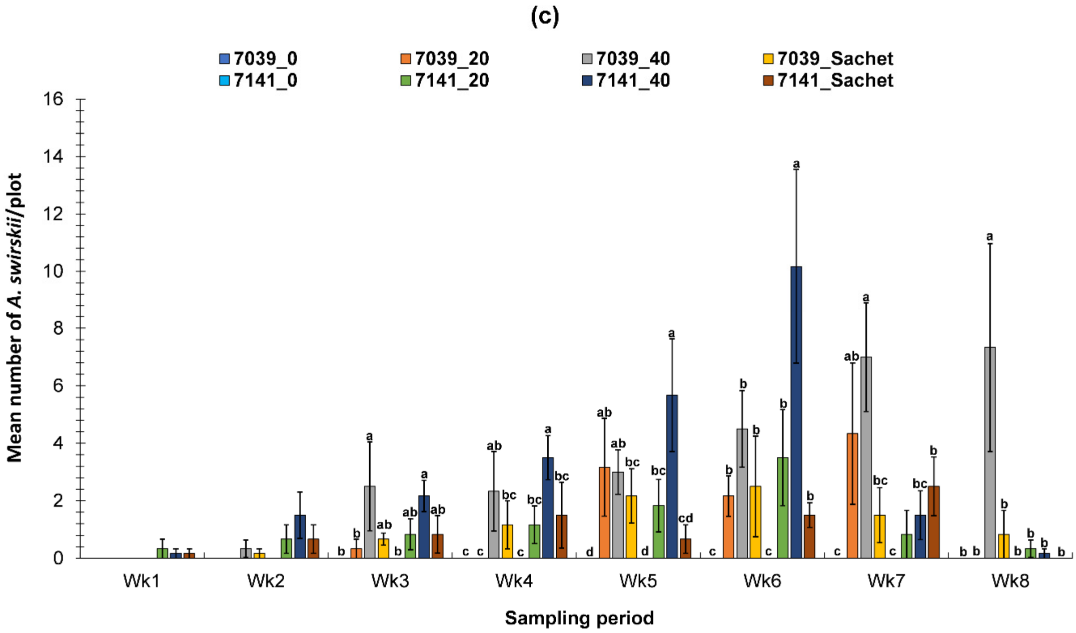


Figure 7. Mean number (±SE) of (**a**) *Bemisia tabaci*, (**b**) *Polyphagotarsonemus latus*, and (**c**) *Amblyseius swirskii* life stages recorded per plot on pepper leaves in various sampling weeks of the third field study (fall season). The first part of the treatment name represents pepper cultivar, and the second part shows the number of *A. swirskii* received per seedling before transplanting in the field. An augmentative release of *A. swirskii* was done after week 3. Treatment bars within a sampling period with different letters are significantly different (Fisher's LSD test, $p < 0.05$).

3.3. Pepper Yield during Two Fall Seasons

During the first fall field season, there was a significant effect of treatment on pepper fruit count ($F_{7,35} = 3.73$; $p = 0.0041$), but not on marketable yield ($F_{7,35} = 2.01$; $p = 0.0816$). Significantly lower fruit counts were observed in treatment 7141_sachet and 7141_0 than other treatments (Table 1). Although the extrapolated yield difference in the treatments with a high rate of direct phytoseiid application 7039_40 and 7141_40, compared to untreated control plots 7039_0 and 7141_0 of respective cultivars were >7000 and >14,000 kg/ha, a significant yield difference was observed only in cultivar 7141 (Table 1). The overall yield difference in the phytoseiid treatments of two pepper cultivars compared to untreated plots of respective cultivars were 11%, 13%, and 3% in treatments 7039_20, 7039_40, and 7039_sachet, and 16%, 24%, and 15% in 7141_20, 7141_40, and 7141_sachet.

Table 1. Mean (± SE) number of pepper fruit and marketable yield harvested per plot during the two fall field studies.

	Fall Season 1			Fall Season 2		
Treatments	**Fruit Count/Plot**	**Yield/Plot (kg)**	**Extrapolated Yield (kg/ha)**	**Fruit Count/Plot**	**Yield/Plot (kg)**	**Extrapolated Yield (kg/ha)**
7039_0	625.3 ± 41.8 ab	125.2 ± 10.3 ab	54,659	514.0 ± 28.4 c	112.0 ± 5.4 c	48,861
7039_20	695.8 ± 35.9 a	140.3 ± 7.7 a	61,190	604.5 ± 23.4 ab	131.0 ± 4.8 b	57,153
7039_40	705.5 ± 34.7 a	143.7 ± 6.2 a	62,714	628.2 ± 29.9 ab	140.2 ± 6.4 ab	61,170
7039_Sachet	623.0 ± 32.7 ab	129.1 ± 6.7 ab	56,322	585.2 ± 20.3 bc	134.1 ± 6.1 ab	58,518
7141_0	489.8 ± 51.4 c	107.9 ± 11.7 b	47,060	406.2 ± 15.9 d	90.0 ± 3.9 d	39,283
7141_20	587.2 ± 37.8 b	128.9 ± 9.7 ab	56,242	619.7 ± 23.2 ab	139.3 ± 5.1 ab	60,754
7141_40	644.2 ± 16.0 ab	142.6 ± 4.4 a	62,199	666.0 ± 41.7 a	148.9 ± 7.0 a	64,970
7141_Sachet	574.8 ± 29.3 c	126.2 ± 7.6 ab	55,075	626.7 ± 18.2 ab	136.2 ± 4.31 ab	59,409

Means within a column followed by different letters are significantly different (Fisher's LSD test, $p < 0.05$).

During the second fall field season, a significant effect of treatment was observed on both pepper fruit count ($F_{7,35} = 11.34$; $p < 0.0001$) and marketable yield ($F_{7,35} = 12.11$; $p < 0.0001$). All the phytoseiid treated plots had a significantly higher fruit count and yield compared to two untreated plots (Table 1). The extrapolated yield difference in the treatments 7039_40 and 7141_40, compared to irrespective untreated control plots were higher than the previous season i.e., >12,000 kg/ha and >25,000 kg/ha (Table 1). The overall yield difference in the phytoseiid treatments of two pepper cultivars compared to untreated plots of respective cultivars were: 15%, 20%, and 17% in treatments 7039_20, 7039_40, and 7039_sachet, and 35%, 40%, and 34% in 7141_20, 7141_40, and 7141_sachet.

4. Discussion

A PIF strategy can be a sustainable pest management option for conventional and organic vegetable growers, specifically engaged in pepper productions. A single application of *A. swirskii* directly applied on seedling transplants (pre-planting) of 7039 and 7141 pepper cultivars was sufficient for a significant reduction in the *B. tabaci* population during fall and spring greenhouse studies compared to the untreated control. Further, a >65% reduction in the *F. occidentalis* larval population was observed in the treatments with the high rate of *A. swirskii* mite during both study seasons. With a few exceptions, no significant difference in the performance of two rates of phytoseiid mites was observed indicating an initial release of 20 mites/plant could help reduce pest populations; however, the 40 mites/plant may result in efficient management of multiple pepper pests under controlled conditions. The carrier material provided initial refuge and supplement necessary for the mite survival and establishment on seedlings. In their study on assessing the effect of pollen in the pre-establishment of *A. swirskii* on pepper plants, Kutuk and Yigit [27] showed significant suppression in the *F. occidentalis* with >30 *A. swirskii* released per plant. They suggested the initial release of plant pollen (pre or at the time of predator release) would provide nourishment and improve the establishment of the *A. swirskii* population in the absence of their prey. This would also prove more cost-effective rather than making multiple applications of the predator during the growing season. Consistent with the current results, Ramakers [13] indicated that the early establishment of phytoseiid mites on pepper seedlings could enhance thrips management under protected cultivation. Other researchers also showed promise in the utility of plant supplement for supporting the phytoseiid mite and/or achieving thrips or whitefly suppression [14,17,26,33,34] on the host, which could prove beneficial in developing a PIF approach targeting pests of different vegetables. Based on these results, PIF has the potential to serve as an essential tool for nursery growers with low input costs for pest management of a single release of the phytoseiid mite.

The abundance of *A. swirskii* in the greenhouse studies was greatly influenced by the availability of food sources. In both seasons, *A. swirskii* populations were low during trial initiation, but with the increased flowering and pest buildup, predatory mite numbers increased over time, peaking towards the end of the experiments. In their previous study, Kumar et al. [26] reported similar population dynamics of *A. swirskii* when applied on hot and sweet peppers. A minimal contamination occurred in the fall (0.7% of high rate seasonal mean) and slightly higher in the spring (3–6% of high rate) which had three times the predatory mite populations. Plausible reasons contributing to contamination include (1) handling error in managing growing plants, (2) *A. swirskii* hitchhiking on adult insects or people, and (3) dropping of pepper flowers containing motile stages from adjacent plots. Although extreme caution was maintained in minimizing any handling error during the second season, dispersal of *A. swirskii* in the untreated control plots could not be avoided in the spring greenhouse trial. In the current study, the primary source of inter-plot dispersal of *A. swirskii* could not be ascertained; however, we assume both direct and/or indirect movement of this tenacious phytoseiid mite would prove a boon for nursery pepper growers.

The field application of the PIF approach was assessed over three pepper growing seasons and is one of the few studies where the biological control potential of *A. swirskii* was evaluated on any vegetable in multi-pest situations in Florida agroecosystems. Results from the field studies showed the impact of planting seasons on the arthropods' abundance and the performance of the PIF approach. Unlike the greenhouse assays, in the field conditions, two applications of *A. swirskii* may be necessary (weather permitting) to ensure their establishment and achieve significant suppression in the pest populations inhabiting different plant parts. As discussed earlier, in the current study, we chose to make an augmentative application of *A. swirskii* after the first season for the fairness in trial comparisons. However, it would be valuable to know if the single application of phytoseiid mite (40, 60, or 80/plant) could deliver similar results to reduce the associated input costs.

Among three phytoseiid mite treatments, plots with the high rate of *A. swirskii* (40 per plant) were consistent in addressing foliage pests, *B. tabaci*, and *P. latus* on two pepper cultivars. However, their efficacy in regulating *F. occidentalis* larvae inhabiting flowers was questionable. This phenomenon could be explained based on the low abundance of *A. swirskii* during the spring season (compared to fall seasons) due to relatively colder weather in mid-Florida affecting their population on pepper transplants. *Amblyseius swirskii* is native to the Mediterranean region and adapted to survive in temperatures between 20 and 32 °C [35]. Another possible reason could be their tendency to prey on readily available food. Such differential predation behavior of *A. swirskii* has been previously reported on field cucumber in Florida [28]. In their study, significant suppression in a *Thrips palmi* Karny population inhabiting cucumber foliage was observed, but *A. swirskii* failed to regulate *Frankliniella schultzei Trybom* present in flowers. The low rate of phytoseiid mites and combination of sachet and banker plant treatments did show some promise, but two treatments were not as consistent in regulating pest populations and are not as field compatible as the direct application of the high rate.

The results of the two fall seasons indicated that the initial application of *A. swirskii* using the PIF approach not only delivered significant suppression in the pest population but also translated to an increased pepper yield. During the first fall field season, there was an increase in marketable yield of 12.8% and 24.3% in the treatment with a high rate of *A. swirskii* for cultivars 7039 and 7141, respectively. In the second season, the yield difference between the two treatments was 20.1% and 39.5%, respectively. Very heavy rains and tropical force winds in the first fall season delayed *A. swirskii* population increases and pest population levels were lower than the second fall season which may have resulted in the lack of differences seen in pepper yield. The higher yield difference during the second fall season could be due to the high infestation of *P. latus* (>3×) and *F. occidentalis* (>5×) populations. Although it is hard to substantiate with the current data, cultivar 7039 appeared to have a higher intrinsic ability to resist *P. latus* damage, which resulted in lower yield differences between two of its treatments for both fall seasons compared to 7141. When the extrapolated yield of two cultivars with the high rate of *A. swirskii* was compared to the average bell pepper yield of Florida in 2018, a >40% yield increase was reported in two pepper cultivars during two fall seasons [36]. Pepper yield (both number and weight) increased in treatments receiving direct application method of *A. swirskii* (especially plants receiving 40 mites); however, numerical measurements do not accurately reflect the increase in fruit quality. *Polyphagotarsonemus latus* damage to fruit was extensive in the untreated control plots and, although some fruit were marketable, the quality was poor (Supplementary Figure S5). Although the results clearly demonstrate the yield benefits of using the PIF approach during fall seasons for the Florida pepper growers, further testing of this technique in fields separated by greater geographical distance is needed.

5. Conclusions

In the past decades, the Florida vegetable industry has been faced with considerable challenges because of the influx and establishment of new invasive pest species in the region. Some of which, such as *F. occidentalis*, *B. tabaci*, *F. schultzei*, *Scirtothrips dorsalis* Hood, and *T. palmi*, pose a continuous risk to vegetable growers [37–43]. Due to their invasiveness, an essential question before pest management professionals is how to manage these pests simultaneously in a sustainable and eco-friendly manner. Thus, to receive broader acceptance of any alternate control strategies, one of the essential requirements is to make it economical and competitive to chemical control strategies. The PIF approach offers one such alternative to conventional and organic vegetable growers of Florida. Results suggested that a single application of *A. swirskii* at pre-planting can help regulate multiple pests affecting pepper under controlled production. In the field, one application at the pre-planting and another release during early growth stages to augment the existing population of phytoseiid mite can help reduce pests and improve crop yield. The PIF is a sustainable management strategy for regulating multiple pests (like broad-spectrum insecticides) without any chemicals. We believe it to be an economically viable option for pepper growers as it would reduce the cost of labor and chemical applications. However, in Florida, its utility for the organic pepper growers could be limited to only fall production seasons due to the pepper weevil *A. eugenii*'s presence in the spring season, and the cooler spring temperatures at transplant are not conducive for *A. swirskii* sustainability. Future studies should focus on implementing the PIF approach in other horticultural crops and integrating it with conventional chemical-based management programs.

Supplementary Materials: The following are available online at http://www.mdpi.com/2071-1050/12/18/7816/s1, Table S1 Generalized linear mixed model statistics for abundance of arthropods in the greenhouse and field trials; Figure S1: *Anthonomus eugenii* damage observed in different pepper plots during spring field production season; Figure S2: Mean number (± SE) of *Frankliniella occidentalis* and *Amblyseius swirskii* observed per plot in pepper flowers during first fall field study. Bars with different lower-case letters shows significant difference among treatments for abundance of *F. occidentalis*, and upper-case letters represents difference between treatments for *A. swirskii*; Figure S3: Mean number (± SE) of *Frankliniella occidentalis* and *Amblyseius swirskii* observed per plot in pepper flowers during spring field study. Treatment bars with different letters are significantly different; Figure S4: Mean number (± SE) of *Frankliniella occidentalis* and *Amblyseius swirskii* observed per plot in pepper flowers during second fall field study. Bars with different lower-case letters shows significant difference among treatments for abundance of *F. occidentalis*, and upper-case letters represents difference between treatments for *A. swirskii*; Figure S5: Pepper fruits from control plot (on top) exhibiting heavy *Polyphagotarsonemus latus* damage, and the treatment with the high rate of 40 *Amblyseius swirskii* per pepper plant (on bottom) during second fall harvest.

Author Contributions: Conceptualization, L.S.O. and C.L.M.; Methodology, C.L.M., L.S.O., and V.K.; Validation, C.L.M. and V.K.; Formal Analysis, L.M.; Investigation, V.K. and C.L.M.; Resources, C.L.M., and L.S.O.; Writing—Original Draft Preparation, V.K.; Writing—Review & Editing, V.K., L.M., and C.L.M.; Visualization, V.K.; Project Administration, C.L.M. and L.S.O.; Funding Acquisition, C.L.M. and L.S.O. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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