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USING INDIGENOUS *AMBLYSEIUS SWIRSKII* AND *PHYTOSEIULUS PERSIMILIS* TO MANAGE PESTS ON PEPPER PLANTS IRRIGATED WITH AQUACULTURE WATER

by
BRIDGET LEIGH IRELAND

A thesis
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BRIDGET LEIGH IRELAND

Approved by:

____

Dr. Imad Saoud, Professor — Advisor
Biology

M. Haidar
____

Dr. Mustapha Haidar, Professor — Member of Committee
Agriculture

____

Dr. Yusuf Abou Jawdah, Professor — Member of Committee
Agriculture

Date of thesis defense: September 10, 2020

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ABSTRACT
OF THE THESIS OF

Bridget Leigh Ireland for Master of Science
Major: Ecosystem Management

Title: Using Indigenous *Amblyseius swirskii* and *Phytoseiulus persimilis* to Manage Pests on Pepper Plants Irrigated with Aquaculture Water

Population growth, limited food production abilities and water quality issues are major concerns for the world today. Adaptions to mainstream traditional agriculture are necessary to ensure the continued health of the world's population and maintain water quality. Some options for more sustainable food production methods include integrated aquaculture-agriculture systems (IAA) to conserve water and integrated pest management (IPM) practices to limit pesticide use.

The present study evaluated the effectiveness of Lebanese-reared biological control agents *Amblyseius swirskii* and *Phytoseiulus persimilis* against common greenhouse pests on pepper plants irrigated with aquaculture water. The study took place at the AUB Advancing Research Enabling Communities (AREC) facility in the Beqaa Valley, Lebanon. Six metal, high-tunnel tunnels were covered in 50 gauge white insect netting. All plants were irrigated with aquaculture effluent and thus not fertilized. IPM practices were employed to manage whiteflies, spider mites, thrips, and aphids three of the tunnels in the study area, and the remaining three tunnels were left untreated and used as a control. Results show that *A. swirskii* was effective in reducing the infestation level of whiteflies and thrips. Additionally, spider mites were maintained below economic thresholds by *P. persimilis* for the 81 days of the study. A tank mix of *Beauveria pseudobassiana* fungus and the natural fermentation product Spinosad, which is used as an organic pesticide, did not significantly reduce aphid populations below economic threshold levels. Nevertheless, all IPM tunnels produced a much higher yield of peppers and healthier plants than the control, indicating that the common agricultural pests whiteflies, spider mites and thrips can be managed with IPM practices in the Beqaa Valley.

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CHAPTER 1

INTRODUCTION

A. Water Security

Global population is expected to reach nine billion people by 2050 (FAO, 2009), placing quite a strain on freshwater resources and food production. Furthermore, the effects of climate change will intensify, also affecting food and fresh water availability. Approximately 3.6 billion people already live in areas that experience water scarcity during at least one month of the year. Climate change will increase water shortages for between 4.8 to 5.7 billion people worldwide during at least one month of the year by 2050 (Engin *et al.*, 2018). These water shortages will affect irrigation and food production. In addition to water-related challenges, current food production will need to increase by 70% to feed the increasing population (FAO, 2009). Consequently, strategies must be developed to grow more food using existing water resources; a "more crop per drop" approach (Seckler 1996).

The issues listed above will strongly affect developing countries, such as Lebanon, that already experience water scarcity and food insecurity. Although Lebanon is considered to have ample precipitation compared to the rest of the MENA region, existing freshwater resources in Lebanon are over-exploited and poorly managed (Bou Jaoude *et al.*, 2014). A significant source of freshwater quality degradation is the agriculture industry (MOE *et al.*, 2011), which uses about 72% of available fresh water in the eastern Mediterranean region

(Abdul-Rahman *et al.*, 2011). One of Lebanon's primary areas of food production is the Beqaa Valley, which has experienced a considerable decline in freshwater quality as a result of frequent agrochemical use. Further compounding the problems, the Beqaa Valley will see a 10-30% decrease in precipitation by 2050 (Verner *et al.*, 2013), resulting in difficulties in securing freshwater resources to produce food. In the coming years, developing countries will need to explore innovative methods that use less water and better manage the quality of existing water to ensure adequate food production.

B. Integrated Aquaculture-Agriculture Systems

Integrated production systems that produce two crops using the same water is one option for increasing global food production without overusing freshwater resources. Integrated aquaculture-agriculture (IAA) systems involve two or more linked farming systems, one of which is aquaculture (Little and Edwards 2003). An integrated system that uses aquaculture water for irrigation has ecological and nutritional benefits in addition to financial advantages for growers. Irrigating crops with fish effluent results in frequent water exchanges, maintaining optimal conditions for the aquacultured species. IAA systems redirect excess and potentially harmful nutrients in the effluent into agricultural crops instead of directly releasing the effluent into waterways or reservoirs (Ghate *et al.* 1993). IAA systems provide enough nutrients for plant growth (Abdul-Rahman *et al.,* 2011) in addition to producing an aquacultured protein source with no additional water consumption. Financial advantages of IAA systems for growers include reduced water costs, increased plant yields, and a marketable aquacultured species (Al-Jaloud *et al.,* 1993).

C. Pesticide Problems

One of the major concerns of large-scale conventional farming and IAA systems is the management of agricultural pests that can cause significant damage to the crops. Agricultural pests are especially problematic in warm and humid monoculture tunnels. In conventional agriculture, pesticides, defined as chemical herbicides, fungicides, and insecticides and other products, are used to mitigate pest damage and to improve productivity and plant health (Sexton *et al.,* 2007). Pesticide use revolutionized modern agriculture by controlling pests, thus improving crop yield and allowing for more productive large-scale agriculture. However, misuse of pesticides may cause considerable toxicity hazards to humans and damage the environment. Moreover, pesticide misuse has made many chemical pesticides less effective with time, as some pests have developed resistance (Sexton *et al.,* 2007). Additionally, pesticides are difficult to use in IAA systems, as they can be harmful to the people working in tunnels and to the nearby aquacultured species. Consequently, maintaining agricultural pest levels in integrated aquaculture-agriculture systems poses novel challenges to growers.

D Integrated Pest Management, Tunnel Pests, and Natural Enemies

Integrated Pest Management (IPM) programs aim to monitor crops and manage pest populations with limited chemical pesticide sprays (Kogan, 1998), promoting an ecosystem approach. A key component of IPM is scouting for pests and determining economic threshold levels, beyond which pest infestation starts affecting returns of the farms. IPM programs evaluate both economic injury levels and the economic thresholds of crops to ensure that the treatment of agricultural pests does not occur until the financial damages caused by the pests surpasses the financial cost of pest management (Sexton *et al*., 2007).

IPM systems utilize a combination of tools against pests. There are four tiers of IPM management practices that can be utilized to manage pests while reducing pesticide sprays. The first IPM tier involves cultural adaptations, such as site selection, hygiene and seed selection. The next level of IPM utilizes physical and mechanical additions to trap pests or block them from entering the growing area. Another physical addition is the intercropping of other plant species among the crop, such as marigold plants that serve as indicator plants for pest populations and banker plants for natural enemies. The next IPM tier includes biological control agents to manage pests instead of chemical means whenever possible. The last part of IPM treatment involves chemical pesticide sprays as a last resort, while considering the potential damage to other arthropod populations in the growing area. (Barratt *et al*., 2018).

The present study relied on biological control to manage populations of whiteflies, spider mites and thrips below economic threshold levels. Biological control is defined as using one organism to manage or reduce the population density of another organism. In IPM, this is usually accomplished by adding specific species of natural enemies or entomopathogenic fungi in the growing area to reduce populations of a specific pest (Bale *et al.*, 2007). Predatory mites *Amblyseius swirskii* and *Phytoseiulus persimilis* are used as biological control for some of the common greenhouse pests. *A. swirskii* is a thrips and whiteflies predator, and *P. persimilis* effectively manages two-spotted spider mites (Calvo *et al.*, 2015). Growers practicing IPM usually purchase natural enemies from commercial operations that ship the biological control agents to the growers. However, Lebanese customs regulations forbid the importation of exotic organisms to preserve Lebanon's natural biodiversity (Farran, 2019). Consequently, farmers are not able to import beneficial organisms from established companies abroad. There are currently no commercial facilities producing natural enemies in Lebanon, as biological control is a relatively new practice for Lebanon. However, the Plant Pathology Laboratory at the American University of Beirut (AUB) is growing predatory arthropods found in Lebanese greenhouses to use in IPM research in local farms. *A. swirskii* and *P. persimilis* grown at AUB are from local wild fauna and thus accepted by Lebanese officials for use in IPM. Moreover, we believe indigenous species would be better suited to Lebanon's climatic conditions than natural enemies that were raised in a different climate.

CHAPTER 2

MATERIALS AND METHODS

A. Date and Location of the Study

The present work was performed during late spring and summer of 2019 at the Advancing Research Enabling Communities Centers (AREC) of the American University of Beirut (AUB) in the North Central Beqaa plain. The Beqaa Valley is a semi-arid plateau in Lebanon, 1000 m above sea level. The area receives little to no rainfall during summer months. The soil is slightly alkaline with a pH of 8.0. It is a clayey, vertic xerochrept, fine-textured alluvium derived from limestone (Ryan *et al*., 1980).

B. Tunnel Construction

The experiment was performed in six high-tunnel structures ($50m^2$ each, 5m by 10m), covered by white, 50 gauge mesh insect-proof netting. The soil in the experiment area was mechanically tilled and mixed with a router tiller before the tunnel-like structures were erected. Wire was stretched over frames lengthwise to support insect netting on the tunnel frames. Insect-proof netting was stretched over the metal frames and secured with clamps and wire. Double doors with latches were erected on the northern sides of the tunnels to better control potential pest entry.

The experiment was comprised of three replicate IPM tunnels and three replicate control tunnels (Fig. 1). All IPM tunnels used a combination of IPM practices and biological control to manage pests. Tunnels on the southwest side of the experiment area were designated as control tunnels to prevent the wind from blowing the natural enemies into the control tunnels from the IPM tunnels. Control tunnels received no pest management treatment.

Figure 1. The six netted tunnels for the integrated aquaculture-agriculture system were divided into three IPM replicates and three control replicates. Netted tunnels on the bottom were the IPM replicates and received IPM treatment. Netted tunnels on the top served as the control tunnels and received no treatment.

C. Aquaculture

All experimental plants were irrigated with aquaculture effluent for the duration of the study. On May 23rd, approximately 1100 sex-reversed, male tilapia, with an average weight of 19 ± 8 g per fish, were stocked in a $45m^3$ pool near the experiment tunnels. This pool provided irrigation water for the plants in an integrated agriculture-aquaculture system. The pool was constructed from black high-density polyethylene (HDPE) and had a central drain. Every week, about 20% of the tank was drained into a nearby apple orchard because irrigation of the experimental plants did not consume enough water to maintain water quality in the tank.

Tilapia were managed for optimal growth. Fish were offered feed at a daily ration of 3% body weight divided into three equal feeding events at 7:30 am, 12:30 pm and 6:30 pm. Total feed was adjusted weekly with increasing fish weight. Feed was spread manually over one-third of the pool's surface at times of feeding.

Aeration for the pool was provided at night using five air diffusers and a 12 V diaphragm air pump connected to a photovoltaic system. An additional submerged 12 V water pump was used to circulate pool water and increase aeration by splashing the water. Both the air diffusers and submerged water pump were utilized nightly from 9 pm to 7 am. Water quality parameters were monitored weekly to ensure optimal conditions for the tilapia. Ammonia, alkalinity, and nitrite concentrations were tested using a HACH Aquaculture Test Kit. A handheld pH meter was used to measure pH. Dissolved oxygen and temperature were measured using an YSI oxygen meter.

D. Irrigation Setup

During irrigation events, water from the pool was pumped to all plants via a drip irrigation system. Three GR sub-lines for irrigation were laid perpendicular to a 2″ main pipe to irrigate each tunnel. The GR lines had holes every 40 cm and were attached to the main pipe with saddles 125 cm apart. GR lines were stretched to the back of tunnels and secured to the poles to ensure continued tension. GR lines were flushed before the start of the study.

E. Planting

Peppers are a common agricultural crop in Lebanon and were chosen for the present work because of their relatively short growing period and suitability for the local climate. Pepper seedlings (37-110 RZ: Robinson Agri, Byblos, Lebanon) were dipped in a solution of 25mL/20L Previcur ® (propamocarb) fungicide before planting on May 22nd. Seedlings were planted 40cm apart on the irrigation lines forming three rows of 22 plants. Seedling roots were planted five cm deep and irrigated one hour after planting with well water. The average height of seedlings was 15.74 cm from soil to top of leaves at the time of planting.

Previously planted, insect free marigolds in plastic pots were placed in the tunnels and served as banker plants for natural enemies and indicator plants for pest populations (Fig. 2). A total of nine potted marigolds were dispersed in each tunnel. Spaghetti tubing was installed from the irrigation lines to irrigate the marigolds with fish effluent at the same time as i the peppers. Dead marigold flower heads were cut and removed from the tunnels frequently to allow for continued flowering throughout the experiment and to remove potential pests from the experimental area.

Figure 2: Rows of pepper plants in netted tunnels with potted marigolds intercropped.

F. Scouting and Data Recording

Twelve plants per tunnel (four per row) were randomly selected and marked with a flag. These plants were used for monitoring throughout the study. Scouting consisted of randomly selecting of leaf from the top, middle, and bottom sections of each plant and examining both sides for pests and natural enemies. When counts of pests per leaf exceeded 200, counting was stopped and the number recorded as 200 for the leaf (600 maximum per plant). Flagged pepper plants were scouted twice a week for adult whiteflies, thrips, spider mites, and aphids beginning 20 days after transplanting. Counts of adult *P. persimilis* and *A. swirskii* on the flagged plants were also recorded.

The twelve flagged plants were additionally monitored for height, flower count, peppers smaller than market sized, and harvested fruit number and weight. Plant height was measured weekly from the soil level to the highest point on the plant. Fully formed and open flowers were also counted weekly and scouted for pests twice a week. Fully formed peppers measuring between 1 cm and 10 cm in length were not considered market size but were counted weekly as a growth parameter. Data was collected for 81 days.

Marigolds were scouted at the same time as the peppers. Marigold flowers were shaken firmly over clean pieces of white paper, and arthropods that dropped off were counted and recorded. Pest counts on both the marigolds and the pepper plants were considered in determining the timing of natural enemy releases.

G. Biological Control Releases

Natural enemies *A. swirskii* and *P. persimilis* reared at the Plant Pathology Laboratory at AUB were released in the IPM tunnels when pest populations exceeded what is considered the economic threshold for peppers in Lebanon. Action thresholds for the current experiment were set at 1 adult whitefly per plant, 5 adult thrips per plant, 10 spider mites per plant, and 3-4 aphids per plant (Schuster and Smith, 2015). Prior to releasing the natural enemies, both species of mites underwent quality control checks. *P. persimilis* were transported to AREC in plastic Ziploc bags on cucumber leaves (Fig. 3). Leaves containing *P. persimilis* were placed in each marigold pot and randomly on top leaves of pepper plants to release the mites. *A. swirskii* were transported to AREC in plastic bottles containing a bran mixture to protect the mites. Mites and bran mixture were sprinkled evenly over the upper leaves of pepper and marigold plants. Releases of *P. persimilis* occurred on July 3rd and July 29th. Releases of *A. swirskii* occurred on June 13th, July 3rd, and July 29th.

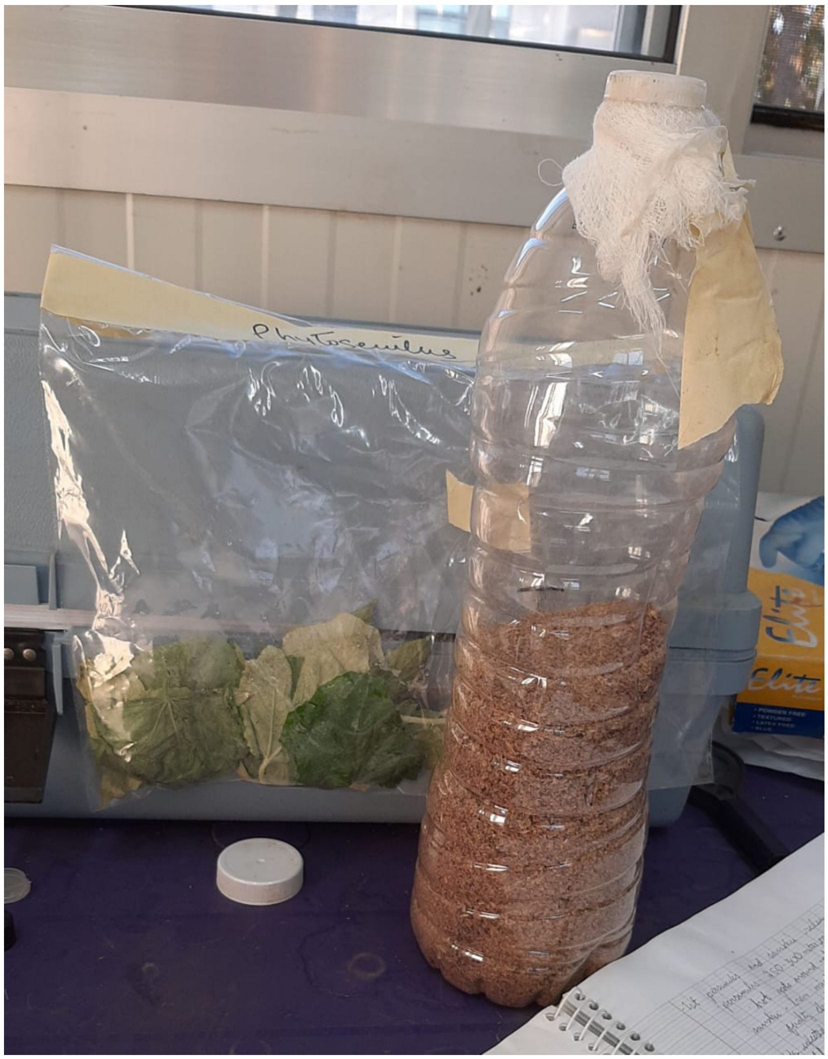


Figure 1: Natural enemies *P. persimilis* are on the cucumber leaves in the plastic bag (left) and *A. swirskii* are in the bran mixture in the plastic water bottle (right) before being released on the pest infested pepper plants.

H. *Beauveria bassiana* Treatment

Aphids are common pests in commercial greenhouse crops in Lebanon. However, no natural enemies for aphids are currently reared commercially in Lebanon. Accordingly, an alternative method of biological control for aphids had to be developed. *Beauveria* sp. is an entomopathogenic fungus used as biological control against thrips, aphids, and whiteflies (Groden, 2009). For the present study, whenever aphids were scouted on a plant in the IPM tunnels, a conidia suspension (conidia 10^8/mL+ emulsifier +1% corn oil + water) of *Beauveria pseudobassiana* grown at the AUB Plant Pathology Laboratory was sprayed on each infected plant and the two plants on both sides of it. Due to the dry environment in the Beqaa plain, *B. pseudobassiana* treatments were applied on plants between 6 pm and 7 pm to allow the mixture to sit on plants overnight without photochemical reactions. *Beauveria* sp. was sprayed using a GDM Gaia 20L backpack sprayer with a cone nozzle during evenings on days with relatively high humidity and low temperature to give the fungus a better chance of survival. *B. pseudobassiana* sprays were performed on July 15th, July 22nd, and August 8th.

I. Spinosad Treatment

Additional management of aphids was deemed necessary in the later stages of the experiment after counts of aphids in the IPM tunnels remained high. Spinsosad, a natural fermentation product approved for use as pesticide in organic agriculture, was sprayed on the pepper plants in the treated tunnels on August 13th to further manage aphids. A mixture of 35mL of very highly concentrated Spinosad in 20L of water was applied uniformly to all

IPM tunnels with a GDM Gaia 20L backpack sprayer with a cone nozzle. Five liters of mixture per greenhouse was applied. Spinosad application occurred after dark when temperatures were lower to maximize the pesticide's efficiency.

J. Harvesting

Peppers fruits from all tunnels were harvested weekly beginning on July 9th. Peppers of marketable size (larger than 10cm) on the flagged plants were removed first and counted separately by plant. All peppers in the tunnel larger than 10cm in length were removed from the plants, counted, weighed and averaged by treatment. A total of six harvests took place over the course of the growing season.

K. Statistical Analysis

Plant growth parameters (plant height, number of flowers and number of peppers smaller than market size), pepper production, and pest infestation were compared between the IPM and control tunnels. The mean number of each type of pest in all IPM and all control tunnels per scouting date was recorded. Scouting dates were averaged by week and plotted per tunnel. Data was analyzed using SAS (V.9.2, SAS Institute Inc., Cary, North Carolina, USA). Differences among treatment means were considered significant at $p < 0.05$.

CHAPTER 3

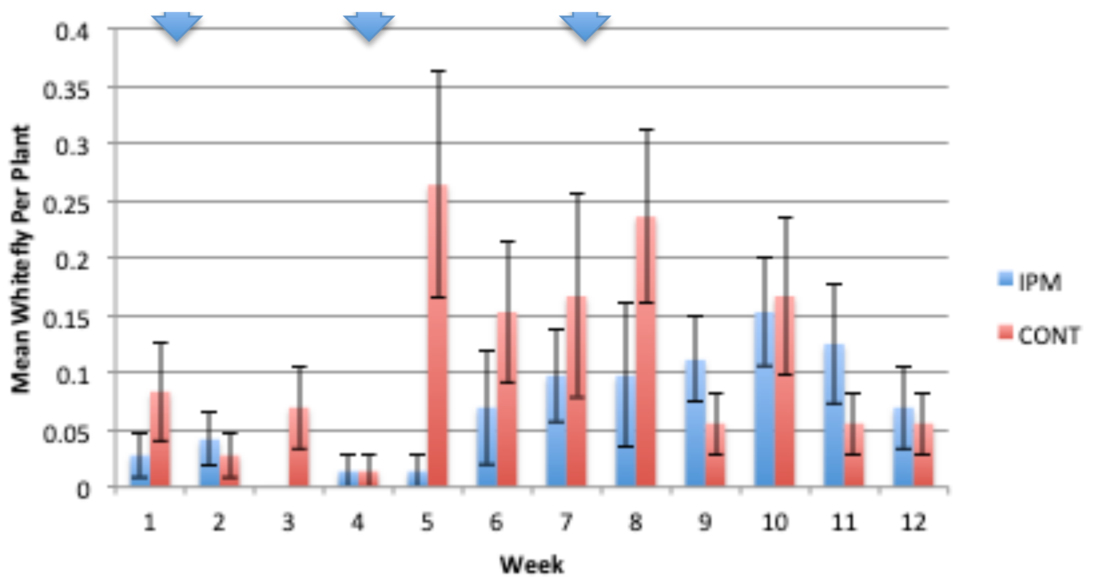
RESULTS

A. Pest Abundance on Pepper Plants

A 1. Whiteflies

Whitefly counts remained relatively low throughout the experiment. Whiteflies were first observed during Week 1 in both the IPM and the control at average densities of less than one whitefly per plant (Fig. 4). Whiteflies densities remained less than 0.3 whiteflies per tunnel in the control tunnels and 0.15 whiteflies per tunnel in the IPM tunnels. In the final weeks, average counts of whiteflies in the three IPM tunnels and the three control tunnels decreased substantially. It is noteworthy that some *A. swirskii* were observed in the control tunnels during the final weeks, suggesting contamination from the IPM tunnels.

Figure 2: Comparison of average whitefly counts per week on the entire plant between IPM and control tunnels. Blue arrows indicate releases of *A. swirskii* in IPM tunnels. Bars indicate standard error.



A 2. Thrips

Thrips were observed in very low numbers on the pepper leaves in both the control and IPM tunnels starting from Week 1 (Fig. 5). The greatest average counts of thrips in the three control tunnels were recorded in Week 8 (2.13 thrips/plant). Greatest recorded average counts of thrips in the IPM were observed in Week 9 (0.96 thrips/plant). The average number of thrips on the plants in the control tunnels exceeded the average number of thrips in the IPM tunnels every week except for weeks 1, 5, and 9.

The average recorded counts of thrips in the pepper flowers tended to be greater than the observed number of thrips on the leaves. Thrips were first recorded in the pepper flowers during Week 2 in both the IPM (0.40 thrips/flower) and control tunnels (0.18 thrips/flower) (Fig. 6). The greatest average thrips counts in flowers in both IPM (6.72 thrips/flower) and control (8.64 thrips/flower) was recorded in Week 6.

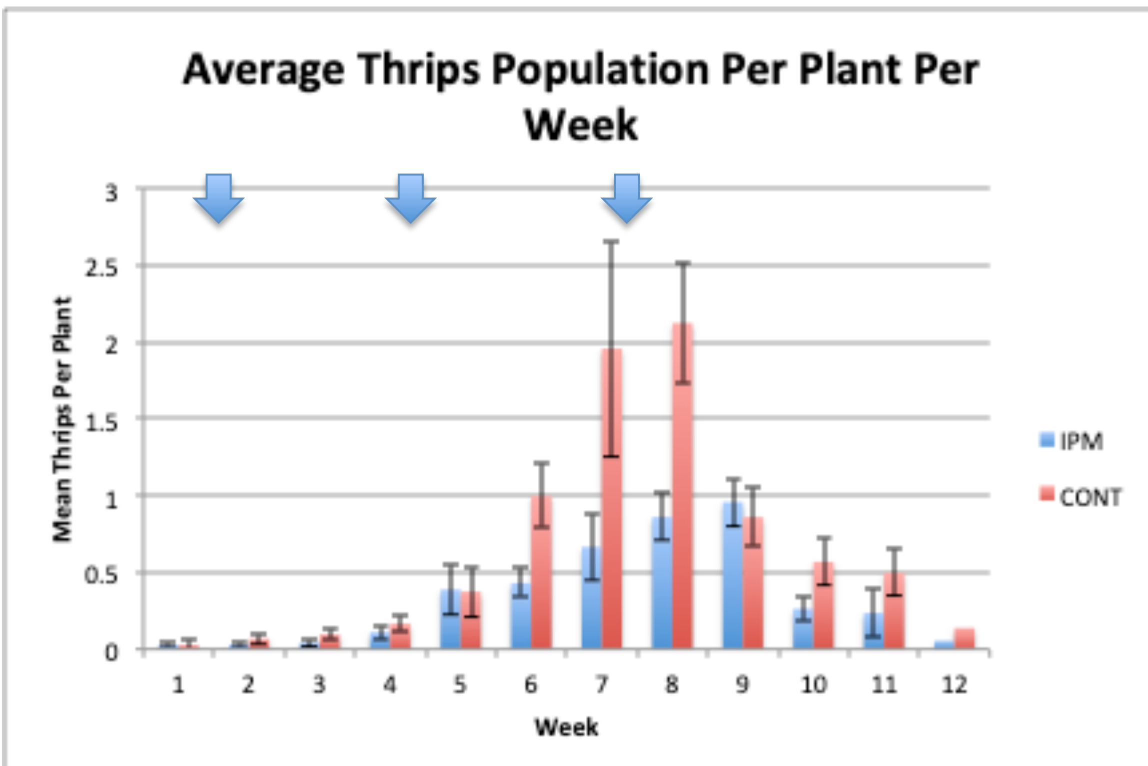


Figure 5: Comparison of average thrips counts per week on the entire plant between IPM and control tunnels. Blue arrows indicate releases of *A. swirskii* in IPM tunnels. Bars indicate standard error.

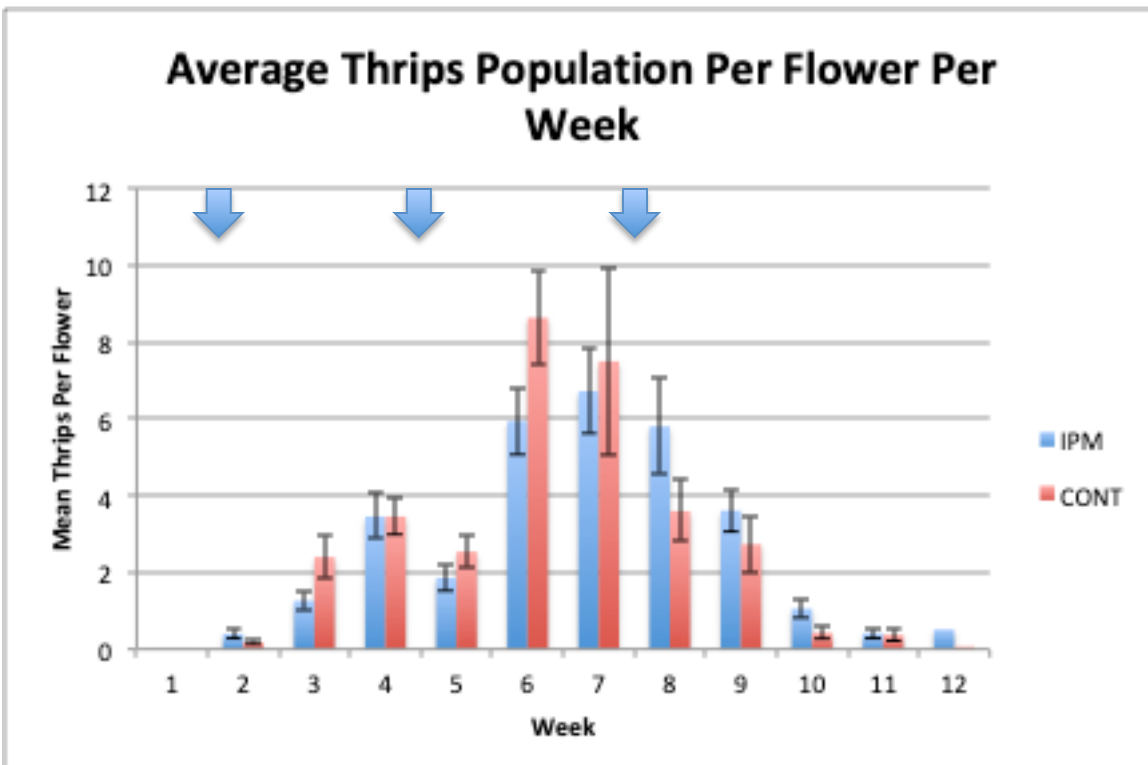


Figure 6: Comparison of average thrips counts per week on the pepper flowers between IPM and control tunnels. Blue arrows indicate releases of *A. swirskii* in IPM tunnels. Bars indicate standard error.

A 3. Aphids

Aphids were observed on the pepper leaves in the control tunnel during the first week of scouting and in the IPM tunnels during the second week (Fig. 7). Aphid counts increased steadily in the control, more than doubling each week until Week 8, thereafter increasing at a slower rate. The greatest number of aphids observed in the control was in Week 12 (355 aphids/plant).

Aphids were observed in low numbers on the leaves in the three IPM tunnels and remained less than 2 aphids per plant until Week 8, after which the aphids increased steadily for the remainder of the experiment. The greatest number of aphids per leaf in the three IPM tunnels was in Week 12. IPM tunnels experienced different rates of aphid infestation. One tunnel did not observe any aphids in it throughout the study. In the two other tunnels, some aphids were observed, but not at densities that were as extreme as the control. Aphid populations were significantly greater in all the three control tunnels than in all of the three IPM tunnels throughout the experiment.

Aphids were first observed in the pepper flowers in the control tunnels in Week 3 and IPM tunnels in Week 5 (Fig. 8). Thereafter, aphid populations in the flowers in the control tunnels increased through Week 6, and then decreased suddenly in Week 7. The greatest average aphid counts in flowers in control tunnels were recorded in Week 8 (88 aphids/flower). Throughout the experiment, aphid populations in flowers were greater in control tunnels than in IPM tunnels. However, in the final week of observations, the counts of aphids in flowers of IPM tunnels (58 aphids per flower) increased to become more than in the control tunnels (30 aphids per flower).

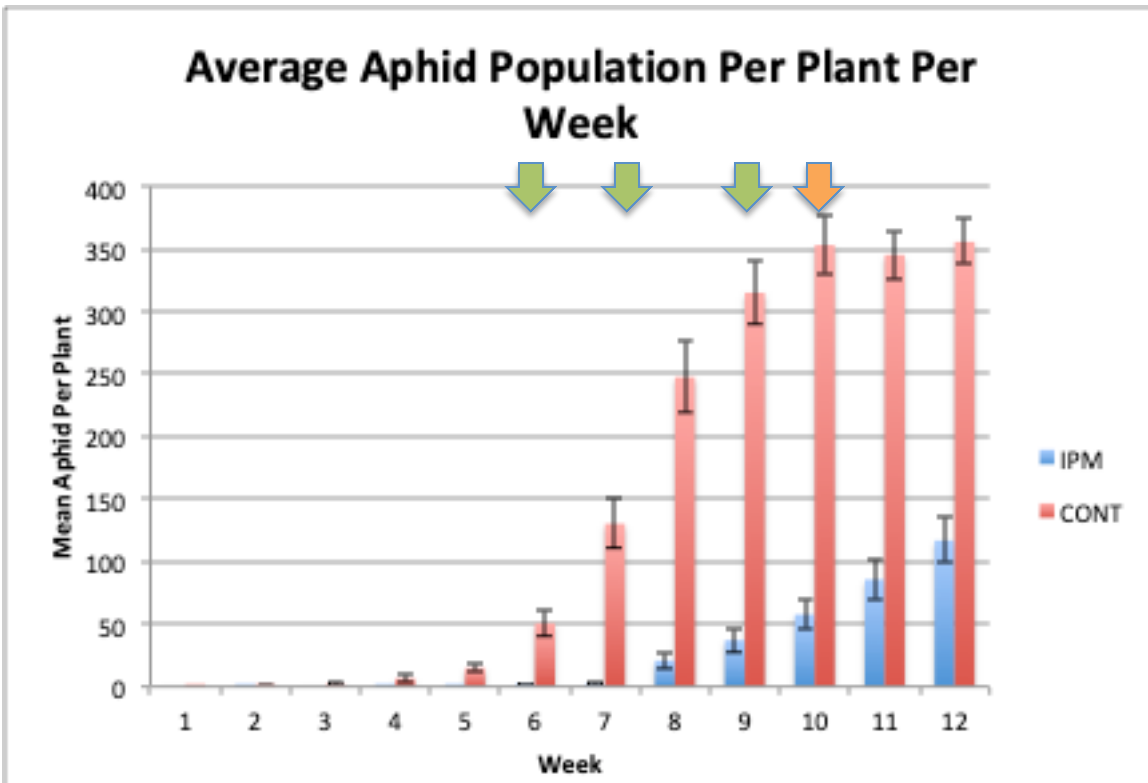


Figure 7: Comparison of average aphids counts per week on the entire plant between IPM and control tunnels. Green arrows indicate sprays of *Beauveria* sp. in IPM tunnels. Orange arrow indicates spray of Spinosad in IPM tunnels. Bars indicate standard error.

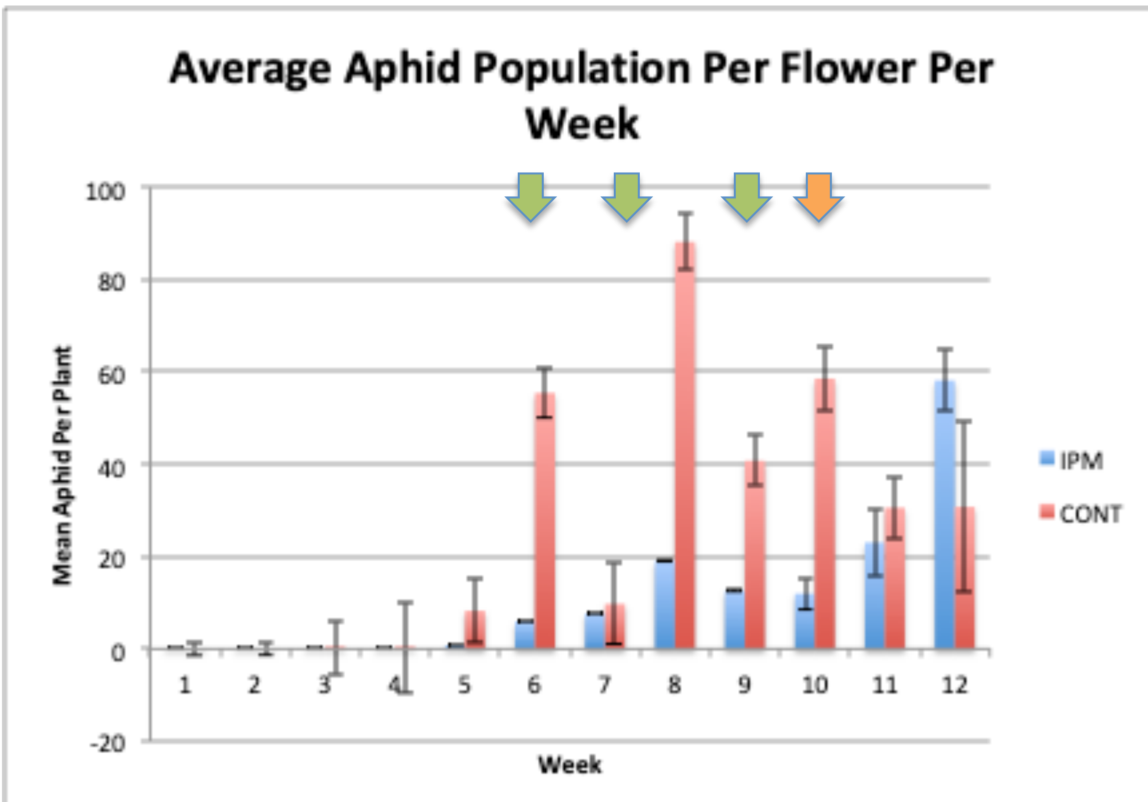


Figure 8: Comparison of average aphid counts per week on the pepper flowers between IPM and control tunnels. Green arrows indicate sprays of *Beauveria* sp. in IPM tunnels. Orange arrow indicates spray of Spinosad in IPM tunnels. Bars indicate standard error.

A 4. Spider Mites

Spider mite populations were first recorded in Week 3 in all tunnels (Fig. 9). The average spider mite counts in the IPM tunnels increased steadily until Week 7. Following release of *P. persimilis* in Week 8, average counts in the IPM tunnels decreased until Week 10, remained steady for Week 11, and increased again in the final week. Average spider mite counts in the control tunnels increased at a slower pace than in the treated tunnels. The highest recorded average spider mite counts in the control tunnels occurred in Week 8 (0.58 mites/plant). Average spider mite counts in the control tunnels decreased after Week 8 until no detections were made in Weeks 11 and 12. Counts of spider mites were greater in the three IPM tunnels than in the three control tunnels during all weeks of the experiment.

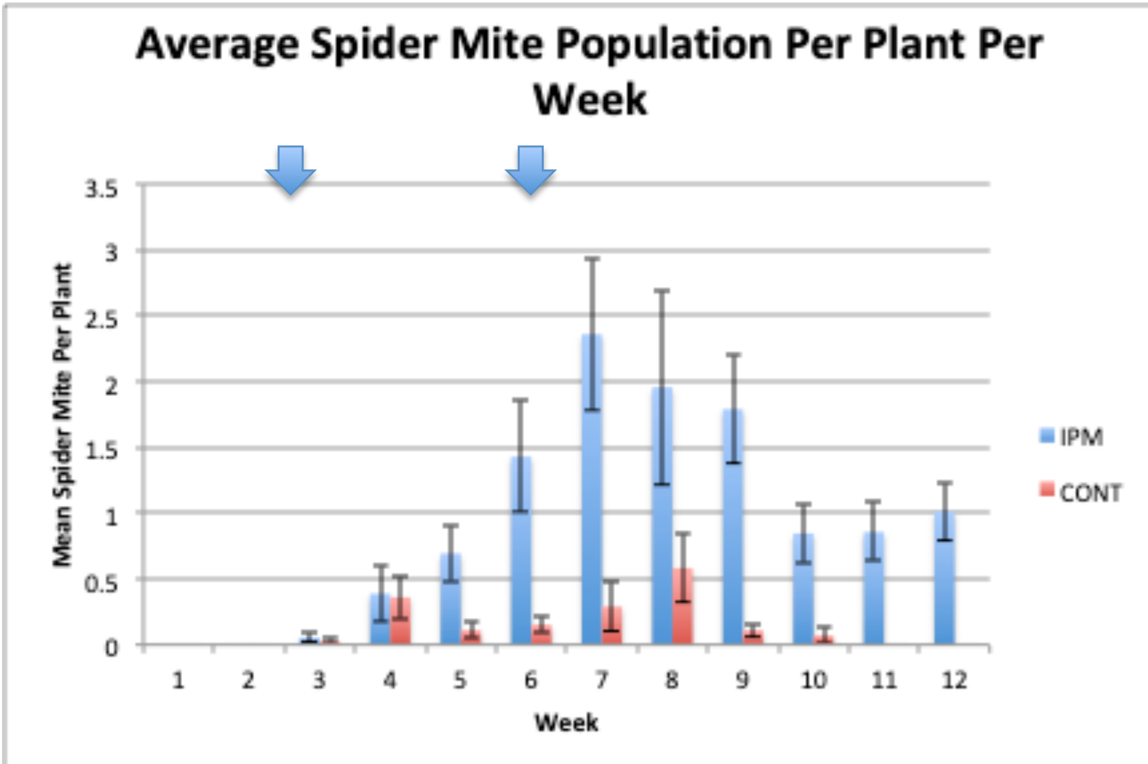


Figure 9. Comparison of average spider mite counts per week on the entire plant between IPM and control tunnels. Blue arrows indicate releases of *P. persimilis* in IPM tunnels. Bars indicate standard error.

B. Release of Beneficial Mites

Releases of beneficial mites occurred when pest populations in the IPM tunnels were at numbers greater than the economic thresholds. Accordingly, three releases of *A. swirskii* occurred on June 13th, July 3rd, and July 29th. Two releases of *P. persimilis* occurred on July 3rd and July 29th. Counts of beneficial mites on pepper leaves remained low throughout the study. The first recorded observation of *A. swirskii* on the leaves of pepper plants in the IPM tunnels occurred in Week 6 (Fig. 10) and greatest counts were recorded in Week 12. Initial observations and greatest counts of *P. persimilis* occurred in the IPM tunnels in Week 7 (0.028 *P. persimilis* per three tunnels) (Fig. 11), then decreased to 0.014 per IPM tunnel in Week 8 and remained at that density until the end of the experiment in Week 12.

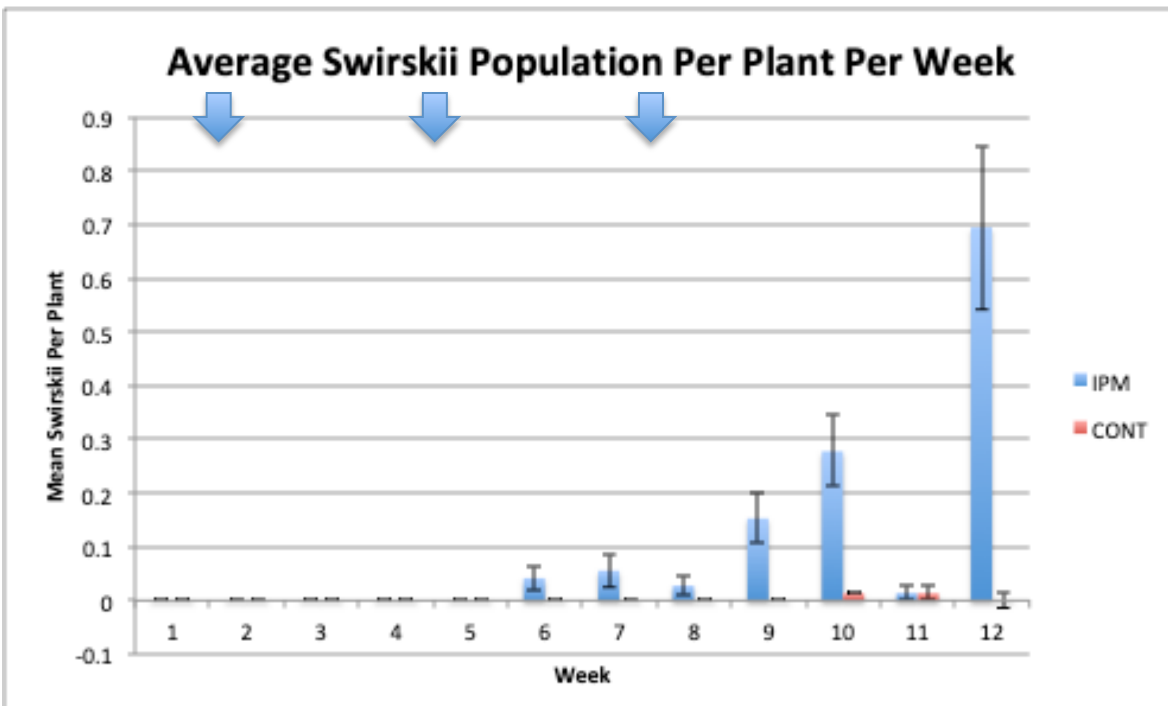


Figure. 10: Comparison of average *A. swirskii* counts per week on the entire plant between treatment and control tunnels. Blue arrows indicate releases of *A. swirskii* in treatment tunnels. Bars indicate standard error.

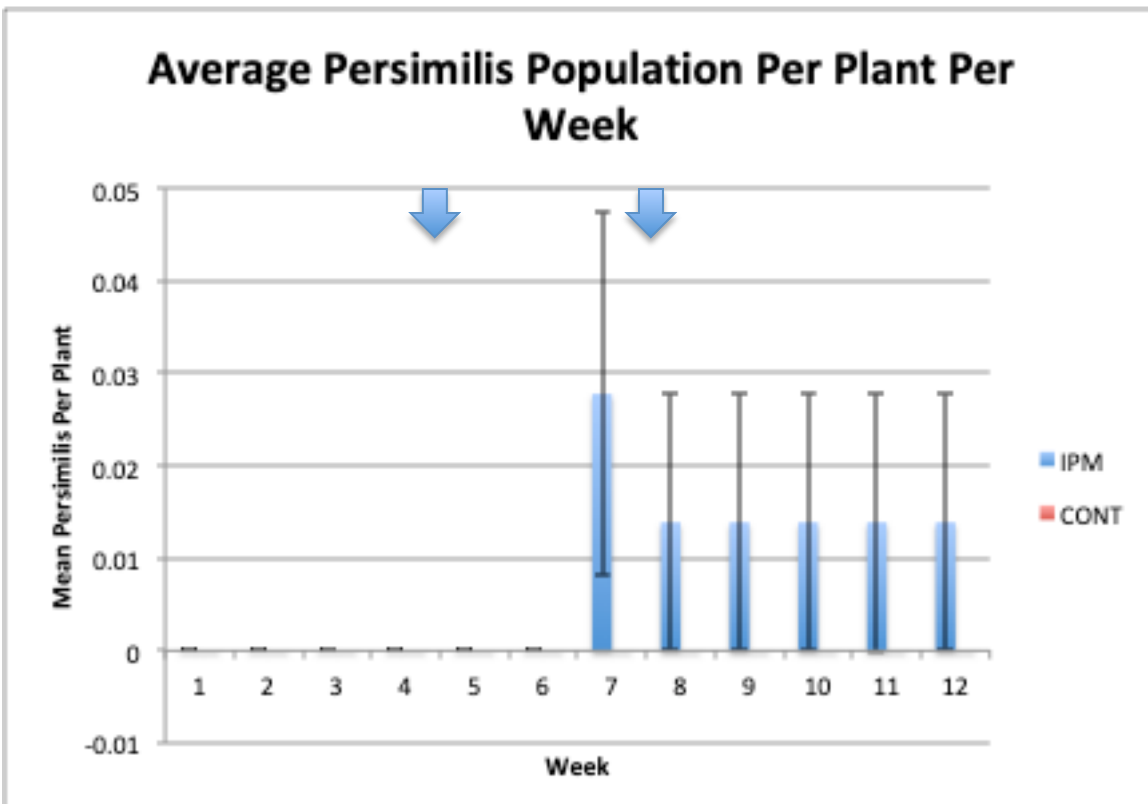


Figure. 11: Comparison of average *P. persimilis* counts per week on the entire plant between treatment and control tunnels. Blue arrows indicate releases of *P. persimilis* in treatment tunnels. Bars indicate standard error.

C. Effect of IPM on Plant Growth and Pepper Yield

Flagged pepper plants were measured weekly to determine the growth rate (Fig.12). During the first week of data collection, plant heights in the IPM tunnels (31.67 ± 0.86 cm) and the control tunnels (31.19 ± 1.13 cm) were similar. There was no significant difference in plant height between the IPM and control tunnels in the final week of the experiment.

Flowers on the pepper plants were first observed in Week 2 in both the IPM and control tunnels (Fig. 13). The average number of flowers in the treated and control tunnels increased steadily until Week 6 when the greatest flower count was recorded in all tunnels.

Peppers that were larger than 1cm but less than 10cm were not considered market sized and were left on the plant until the subsequent harvest. In almost every week, there were higher counts of smaller peppers in the IPM than the control, suggesting more productivity in the treated tunnels (Fig. 14). Peppers were first observed in Week 3 in all tunnels. The average counts of peppers in the IPM tunnels increased steadily through Week 8 (27.31 peppers per plant) then decreased slightly in Week 9 (22.75 peppers per plant). Greatest average counts for both IPM and control tunnels were recorded in Week 10, averaging 34.75 and 28.89 peppers per plant, respectively.

Market size peppers were harvested six times between Week 5 and Week 12 (Table 1). During the first harvest, a greater quantity of peppers was harvested from the three treated tunnels (11.67 kg of peppers per three tunnels) than the three control tunnels (6.74 kg of peppers per three tunnels). In all weeks, more peppers were harvested from the three treated tunnels than the three control tunnels. Greatest average yields from the IPM tunnels occurred in the 5th harvest (93.06 kg of peppers). Greatest average yields for the control (39.60 kg of peppers) were harvested in the 4th harvest. The largest difference between the treated and the control tunnels occurred in the final harvest, 85.54 kg of peppers from the treated tunnels, and 21.19 kg of peppers from the control tunnels respectively

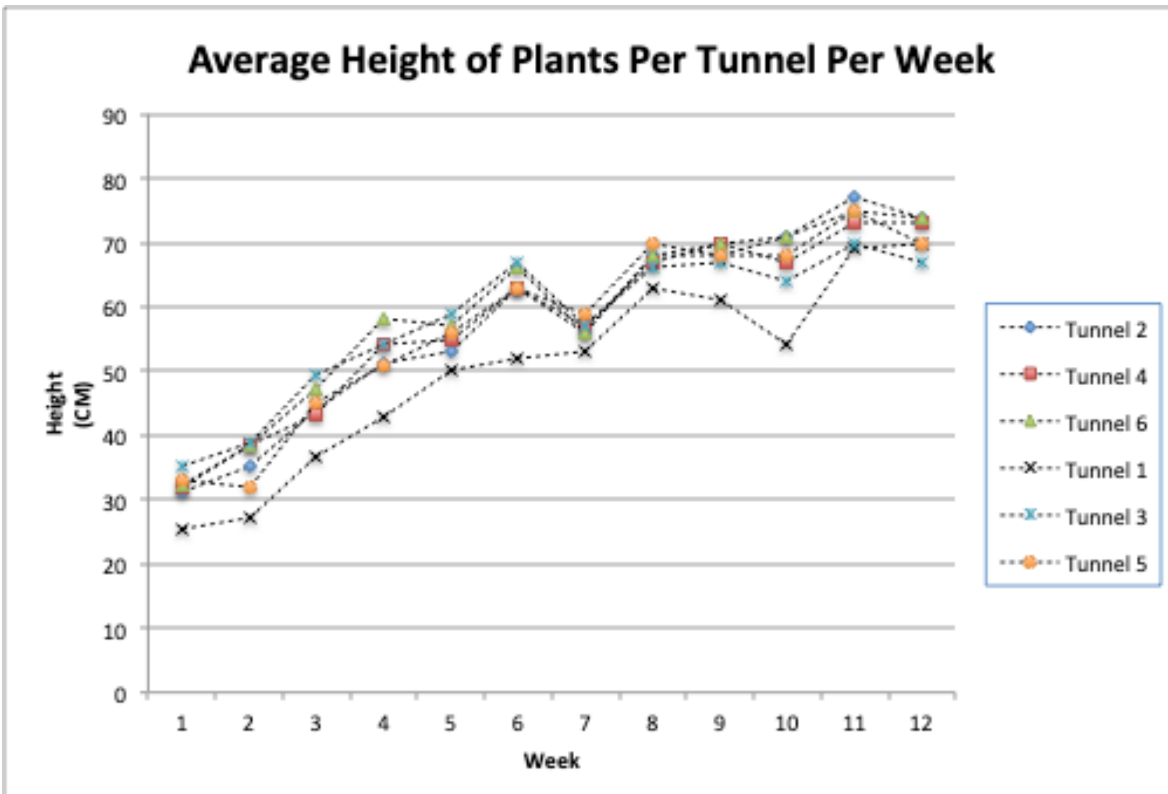


Figure 12: Weekly height of pepper plants per tunnel, measured from the soil to the tip of the highest leaf.

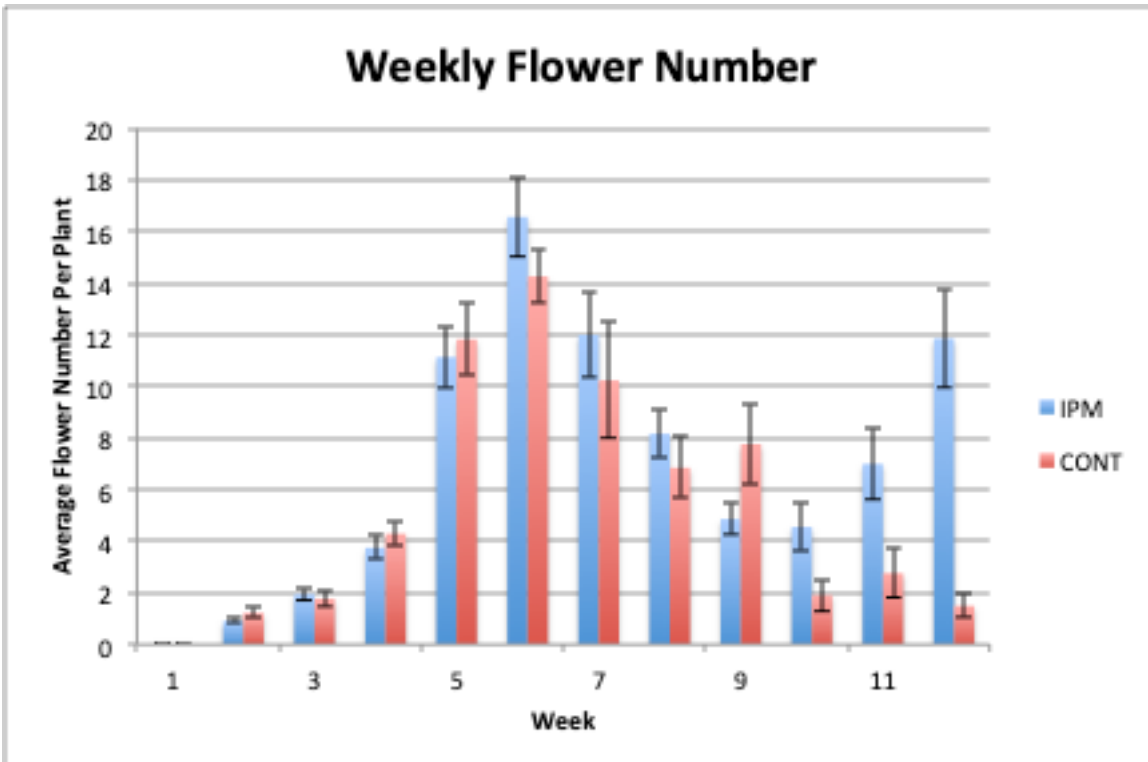


Fig. 13. Weekly average counts of fully formed and open flowers on the plants. Bars indicate standard error.

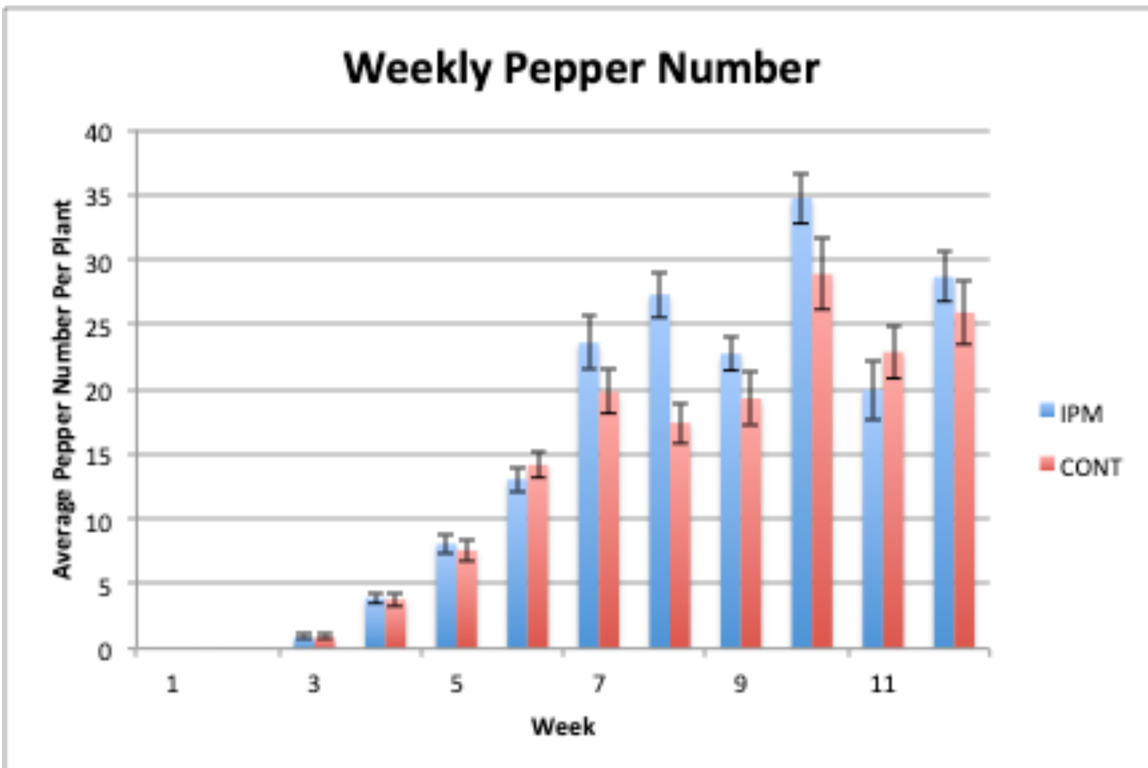


Fig. 14: Weekly number of peppers on plants. Peppers were counted if they were fully formed and longer than 1 cm in length, but not yet marketable length. Bars indicate standard error.

	IPM Tunnels				Control Tunnels			
Week	**Average Number of Peppers per Plant**	**Average Weight of Individual Pepper (g)**	**Total Weight per Harvest (kg)**	**Cumulative Harvest Weight (kg)**	**Average Number of Peppers per Plant**	**Average Weight of Individual Pepper (g)**	**Total Weight per Harvest (kg)**	**Cumulative Harvest Weight (kg)**
1	3.7	15.4	11.7	**11.7**	2.5	14.9	6.7	**6.7**
2	8.0	14.5	25.7	**37.4**	6.5	18.6	16.2	**23.0**
3	9.7	12.5	30.5	**68.0**	6.0	13.2	27.1	**50.1**
4	15.4	17.5	84.8	**152.8**	12.6	14.7	39.6	**89.7**
5	18.5	19.2	93.1	**245.8**	7.0	16.4	38.7	**128.3**
6	12.5	20.1	85.5	**331.4**	6.0	11.7	21.2	**149.5**

Table 1: Harvest results, including the average number of peppers per plant, the average weight of each pepper (g), the total harvest weight per week (kg), and the cumulative harvest for the season (kg).

F. Climate Data

Minimum temperature during the experiment was recorded at 7º C on the night of May 26th (Fig. 15). The maximum temperature recorded was 38º C on June 26th, 2019. Relative humidity was recorded at a minimum of 6.66 on July 22nd, and a maximum of 108 on June15th, 2019 (Fig. 16).

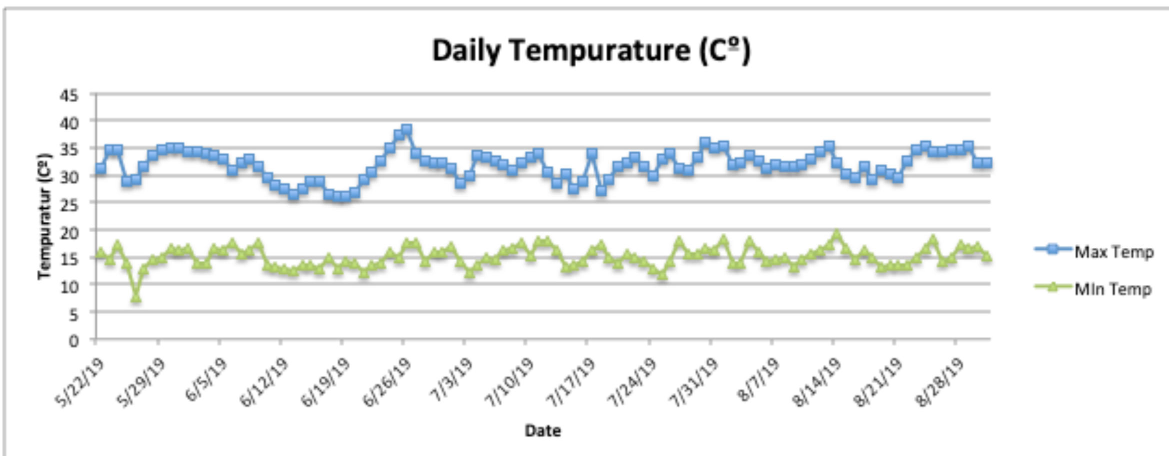


Figure 15: Maximum and minimum temperature (Cº) recorded between May 22nd and August 30th, 2019, at the AREC facility in the central Bekaa Valley, Lebanon.

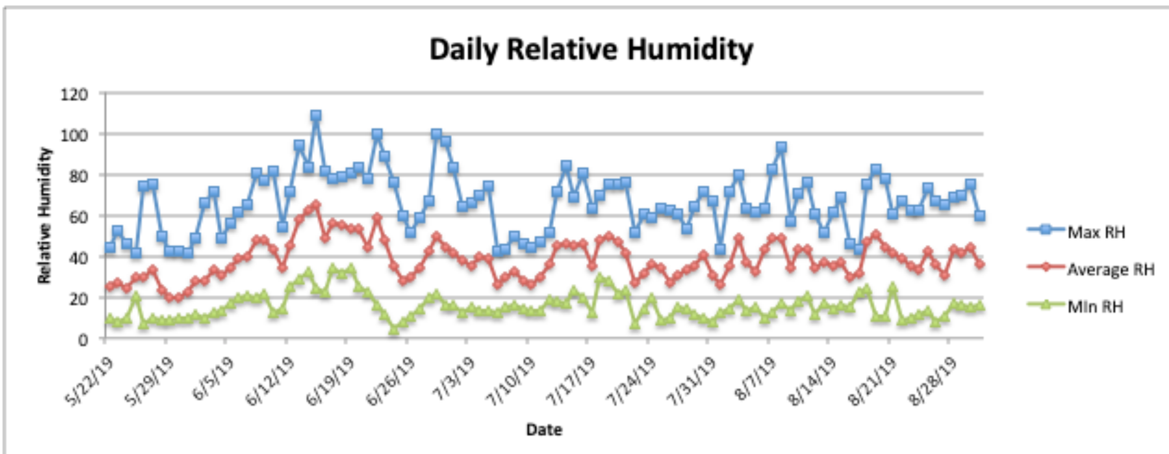


Figure 16: Maximum, average, and minimum relative humidity recorded between May 22nd and August 30th, 2019, at the AREC facility in the central Bekaa Valley, Lebanon.

CHAPTER 4

DISCUSSION

A. Pest Populations and Interactions

High counts of aphids were a consistent issue in the present study, affecting populations of both spider mites and whiteflies in all tunnels. We believe that the heavy aphid infestation on plants in the control tunnels did not allow for the establishment of spider mites or whiteflies. The data shows consistently aphid populations were constantly greater in the control tunnels compared to the treated tunnel, and greater counts of spider mites in the IPM tunnels compared to the control tunnels. Aphid infestations caused an increase of sticky honeydew on the pepper leaves, and spider mites prefer feeding and laying eggs on leaves that are not covered with honeydew (Cédola *et al.*, 2013). This could explain why spider mites established better in the IPM tunnels that had less aphid infestation.

Whitefly populations have been shown to increase slower in areas where there are many aphids. Aphid infestations on chili peppers cause an immune system response in the plants that release higher levels of volatile organic compounds (VOCs), which make the plant less attractive to whiteflies (Saad *et al.,* 2015). Female whiteflies have been observed to have an olfactory preference of VOCs emitted from non-aphid infested chili plants compared to VOCs emitted by plants damaged by aphids. Consequently, female whiteflies tend to settle on plants not infested by aphids.

Detrimental effects of aphid infestation on the health and productivity of pepper plants in control tunnels were increasingly obvious in the final weeks of the study. Aphid infestation is known to cause flower and fruit abortion in peppers according to Frantz *et al.,* (2004). Peak average flower counts in both the treated and control tunnels were observed in Week 6, after which flowers in control tunnels declined considerably concomitantly with an increase in aphid population. Leaves and flowers in the control tunnels had high amounts of sticky honeydew covering them, which likely prevented the development and opening of flowers. During the final four weeks of the trial, leaves began to drop from plants in the control tunnels, flower growth halted, and remaining pepper fruits stopped growing and began to turn red.

Our results on the damaging effects of aphid populations on pepper growth are further supported by the fact that in the IPM tunnels, increased aphid numbers in the final weeks of the experiment caused a substantial decrease in yield. However, this decrease was not equal across all treated tunnels. In one of the three treated tunnels, aphid populations were not observed, and both the fruit weight and number increased from the previous week. A second IPM tunnel displayed moderate aphid infestation in approximately half of the plants during the final weeks, and had only a small increase in the final harvest. The third IPM tunnel also had moderate counts of aphids per plant, but the aphids were found on all the plants. The final fruit yield in this tunnel was less than that of the previous week. All observations strongly suggest a negative correlation between aphid infestation and pepper fruit yield.

B. Management of Aphids

As mentioned previously, there are no commercial natural enemies for aphids available in Lebanon. Accordingly, we resorted to the utilization of alternative IPM solutions such as spraying plants with *Beauveria* sp. and Spinosad. However, the high temperature and low humidity of the Beqaa Valley appears to have decreased the effectiveness of *Beauveria* sp. *Beauveria* sp. appeared to suppress the growth of aphid one to two weeks following the application on the treated plant leaves, but was not successful in the long run in reducing population numbers. Following the sprays of *Beauveria* sp., there was no observation of the white fungal growth on the dead aphids that is characteristic of successful *Beauveria* sp. infection, most probably due to the prevailing dry conditions. Additionally, significant proportions of the living aphids scouted on the treated leaves appeared unharmed and were alive. Previous observations show that the local Beauveria isolate suppresses low densities of aphid populations more effectively than higher densities and thus should be used at the beginning of infestation; furthermore, its efficacy is greatly reduced as temperatures exceed 28 ºC (Abou Jawdeh, 2019). Optimum relative humidity for *Beauveria* sp. isolates has been estimated to be 60% at high temperatures (PC and Padmaja, 2010). We know that maximum temperatures during the study period were greater than 28 ºC and average relative humidity was less than 60% on the majority of the days.

Even though three successive *Beauveria* sp. sprays reduced aphid population growth as compared to the control, their efficacy was not enough to reduce the population of aphids to below the economic threshold level. Accordingly, Spinosad was utilized for additional management of aphid populations later in the study. Similarly to the effects of *Beauveria* sp., growth of aphid populations following the spray of Spinosad slowed slightly, but did not decline significantly. The experiment was terminated before repeated Spinosad sprays occurred and thus management of aphids remained an unresolved challenge during the present study

Management practices for aphids had consequences on the survival of thrips and beneficial mite populations in the IPM tunnels. *Beauveria* sp. and Spinosad are generally effective in managing thrips in addition to aphids (Smith, 2015). *Beauveria* sp. and Spinosad sprays were targeted at the leaves of the pepper plants to avoid damaging the flowers. However, the spraying of Spinosad in Week 10 is correlated with a sharp decrease in observed thrips populations in the flowers in the treated tunnels during the final weeks of data. Additionally, *A. swirskii* populations were observed to decline considerably in Week 11 following the Spinosad spray. Although Spinosad is authorized for use in organic farming (Fernández *et al.*, 2017), our observations in the present study indicate that Spinosad may damage the health and survival abilities of natural enemies on the plants when applied to manage aphids.

C. Beneficial Mites

The beneficial mites *A. swirskii* and *P. persimilis* utilized in this study successfully maintained whitefly, thrips, and spider mites populations below their respective economic thresholds in the treated tunnels, despite low observed counts of the natural enemies. There was an observed decline in pest populations in the IPM tunnels in the two to three weeks following the release of natural enemies.

It is important to note that high temperatures and low relative humidity during the experiment did negatively affect the pest management and survival abilities of released natural enemies *P. persimilis* and *A. swirskii*. In countries where biological pest control is prevalent, *P. persimilis* has a long history of successfully controlling spider mites and is one of the oldest biological control agents commercially available. However, strains used in Europe and North America do not perform well in climates with high temperatures and low humidity (Lenteren and Woets, 1988), such as the Beqaa Valley. Optimal conditions for commonly used *P. persimilis* are between 15º-25ºC (Biological Services, 2020). *P. persimilis* eggs shrivel in low humidity, making successful reproduction difficult in conditions such as those of the Beqaa Valley (Phytoseiulus-System Technical Sheet, 2020). Furthermore, optimum conditions for *A. swirskii* are between 25.6 to 27.8ºC and relative humidity of 70%, (Green Methods Integrated Pest Management, 2020). In the present work, we used indigenous strains of the arthropods and selected for heat tolerance during the rearing process. The fact that we continued to find some of the arthropods in our IPM tunnels several days after release suggests that locally sourced strains can be selected to better tolerate local hot and dry conditions.

In the absence of other food sources, *A. swirskii* and *P. persimilis* have both been known to engage in intraguild predation and consume various stages of other beneficial mites. *A. swirskii* tends to consume *P. persimilis* eggs, larvae and nymphs during times when spider mites are lacking (Maleknia *et. al,* 2016), evidenced by the lower counts of *P. persimilis* towards the conclusion of the study compared to what was applied on the plants. The combination of intraguild predation and climatic factors likely resulted in low numbers of both beneficial mites throughout the study.

D. Marigolds

Marigolds have multiple uses in IPM as a trap plant for thrips and spider mites (Glenister, 2008), a deterrent for whiteflies (Conboy *et al.,* 2019), and a food source for beneficial mites (Maleknia *et al.*, 2016). Spider mites are typically more attracted to the pollen of flowering marigolds than the leaves or flowers of pepper plants (Reddy, 2017). During the present study, observed counts of spider mites during biweekly scouting of the marigolds tended to exceed counts of spider mites on the pepper leaves and flowers in both the control and the treated tunnels. Intercropping of marigolds within pepper plants in the tunnels likely also deterred whiteflies from the pepper plants. Volatile organic compounds from marigolds have been shown to reduce whitefly populations in plants surrounding marigolds compared to areas without marigolds (Conboy *et al.,* 2019). Lastly, marigolds serve as a habitat and a food source for *A. swirskii* in times when thrips and whitefly populations are lacking, allowing *A. swirskii* to manage the low population of these pests over a long term (Maleknia *et al.*, 2016).

E. Recommendations

Growers in the Beqaa Valley of Lebanon frequently employ bad management practices by heavily utilizing pesticides without understanding specific pesticide toxicity or following the recommended application protocols. Growers attempt to manage pests by applying pesticides at first sight to avoid large-scale crop damage and loss from the pests. Additionally, many farmers have frequently reported the use of banned pesticides (Salameh *et al.,* 2004). Accordingly, promoting integrated pest management practices with alternatives to chemical pesticides that are accessible to farmers would have considerable financial, ecological, and health benefits for growers in the Beqaa Valley.

Aphids remained a considerable issue during this study, and were not able to be managed by the IPM methods utilized. For future studies, it may be recommended to try Azadirachtin (neem oil) in organic production systems. In IPM systems, sprays with pirimicarb, or application of neonicotinoids through the irrigation system are recommended in order to prevent significant damage to the natural enemies. In the future, development of natural enemies to target aphid populations will need to be developed and tested.

Alternatives to pesticide use in Lebanon are critical to health maintenance efforts and to preserving water quality in remaining freshwater resources. However, Lebanon faces many social and governmental obstacles to the widespread adoption of IPM practices and management with biological control. Successful biological control requires initial training in scouting methods, proper identification of pests, rearing stations for beneficial organisms and knowledge of ecologically sound pest management methods. To successfully implement IPM programs by Lebanese farmers, considerable extension support for the farmers is crucial.

There will be a considerable process to ensure that biological control can be a feasible option for growers in Lebanon. For proper biological control to be accepted and adopted by farmers in Lebanon, there is a need to produce all necessary beneficial organisms, run demonstration programs that can also be used to train farmers, and have well-trained extension services to assist growers. Efforts should be made to ensure future development prioritizes native biological control agents to avoid competition between native species and released natural enemies.

CHAPTER 5

CONCLUSION

In the present study, an integrated aquaculture-agriculture system was utilized to sustainably produce peppers in the Beqaa Valley by managing pest populations with biological control. This sustainable system allowed for two crops to be produced with the same amount of water and considerably reduced the need for pesticide sprays. Irrigating pepper plants with aquaculture water resulted in a good yield without the use of fertilizers. Using beneficial arthropods controlled some pests nearly as well as pesticides. Present results suggested that IPM could replace traditional agricultural methods without significant decrease in productivity. Lebanese reared predatory mites *A. swirskii* and *P. persimilis* were effective in maintaining pest populations of thrips, spider mites, and whiteflies in IPM tunnels below their respective economic threshold levels. Repeated releases of beneficial predatory mites in IPM tunnels resulted in healthier pepper plants, longer flowering periods for pepper plants, and higher yields.

Tunnels that were not treated with predatory mites and the entomopathogenic fungus were not as productive as the IPM tunnels. Aphid populations were difficult to manage in the IPM because at present, no specialized aphid parasites or predators are reared in Lebanon. Treatments by *B. pseudobassiana* and Spinosad reduced aphid populations but were not sufficiently efficient in maintaining aphid populations below harmful levels. Continued studies of local natural enemies in Lebanon and the development of successful IPM services will be critical in introducing alternatives to conventional agricultural practices, especially if coupled with IAA for water conservation.

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