

Organic Farming Research Foundation 2010

Fungi, Predatory Mites and Guardian Plants for Thrips IPM in Organic Greenhouse Ornamentals

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1. Project Summary

This project tested a novel approach for integrated pest management (IPM) of thrips in a commercial greenhouse of organically-grown spring bedding plants, combining predatory mites, a granular formulation of an insect-killing fungus and marigolds into one effective “guardian plant” system (GPS). The assumption was that adult thrips will be attracted out of the crop to the flowering marigolds, where they will feed and reproduce. Immature thrips will serve as prey for the predatory mite, *Neoseiulus cucumeris*, sustaining them and encouraging their dispersal throughout the crop. Thrips escaping predation will drop to the soil to pupate, where they will become infected with the fungus. The granular formulation will enable the fungus to colonize the potting mix, eliminating the need for reapplication. This represents a low-cost, organic approach, achieving thrips IPM through a holistic system: ATTRACT, SUSTAIN & KILL. Because fungal treatments and mite releases are applied to the guardian plants rather than the entire crop, management costs are reduced while control is maximized.

The marigold GPS was tested in a greenhouse of organic vegetables and compared with two other treatments in separate greenhouses: 1) GPS: marigolds with predatory mites and fungi, 2) IND: marigolds with predatory mites only; 3) Control: no marigolds or biological controls. For the GPS treatment, slow-release sachets of predatory mites were hung on the marigolds and replaced once during the 12 wk long experiment. The potting mix was treated with a granular fungal formulation once before placing plants in the greenhouse. For the IND treatment, predatory mite sachets only were used on the marigold. For 12 wks the number of thrips and mites on yellow sticky cards, marigold GPS and a random selection of crop plants were counted biweekly. Flower samples from the GPS were taken after 6 and 12 wks to count predatory mite and thrips populations. Thrips samples were tested for fungal infection at the end of the experiment.

Thrips populations on the marigolds were higher in the IND than in the GPS greenhouse. More mites and thrips were present in marigold blossoms than on foliage. Lower numbers of thrips were found in blossoms from the combined fungus/mite treatment. Thrips numbers were lower overall on sticky traps in the GPS greenhouse where the combined fungus/mite treatment was tested than in the IND house with mites only. Thrips were found to be infected with fungus at the end of the experiment and fungal growth on the soil surface was evident throughout the experiment. These results demonstrate the potential of the marigold GPS as a useful tool for thrips management in organic greenhouses.

2. Introduction

This project addressed a serious pest of organic greenhouse production nationally. Thrips are the most common reason organic growers suspend organic certification in their greenhouse crops, fearing the loss of their entire crop to this persistent, virus-transmitting pest. Even growers who rely on chemical control find the standard insecticides ineffective due to resistant pest populations. Biological control approaches for thrips will directly benefit organic producers, but will also meet the need of traditional growers who seek to produce plants more ecologically. The innovative IPM strategy we tested could offer economic benefits to growers by reducing thrips damage, increasing plant quality and minimizing production costs by providing a sustained source of biological controls in the crop. It builds on our research over the past two years supported by the USDA and national grower associations to assess marigolds as indicator plants. We found marigolds were highly attractive to thrips and were an effective monitoring tool, but they also became a reservoir for the pest. We project the marigold guardian plant system will both supply sustained thrips biological control and serve as an effective early detection tool for greenhouse pests. Because we are conducting this work in a commercial bedding plant crop, our results will directly apply to the “real world” and will be readily adoptable by other growers.

Spring bedding plants and ornamentals represent a significant revenue source for many organic vegetable growers in New England. Growers can make as much income from these ornamentals in two months as they do over an entire summer of selling vegetables (J. Manix, VT grower, pers. com.). While many customers may quibble over an extra 10 cents for an ear of organic corn, they will not hesitate to pay whatever it costs for a flowering plant to beautify their yard or starter vegetable plant for their garden. Ornamentals are an essential revenue component for sustaining organic growers, without which many farms would not be economically viable. However, organic growers commonly suspend their organic practices in the production of ornamentals and bedding plants because of persistent pest problems, in particular thrips. This project tested a system that could revolutionize pest management, providing growers with an effective, affordable approach to thrips IPM. Growers would be eager to expand their organic production to greenhouse ornamentals if systems were developed that demonstrated success. In addition, growers that follow traditional chemical-based practices find thrips management difficult. They would likely adopt this approach if it were shown to be as or more effective than chemical control. Therefore, though this project specifically targets organic grower needs, the results will also benefit non-organic growers who seek to achieve more ecological pest management.



Figure 1: Marigold GPS in organic commercial greenhouse onion crop.

Current recommended biological control strategies for this pest, which commonly require weekly releases, are expensive for small growers, especially given that weekly freight charges often cost more than the natural enemies themselves. Because thrips populations can increase rapidly, it is often difficult for biological control agents to keep up with the pest. If we are to expect growers to adopt organic practices, they must work reliably over a range of production conditions. Given the narrow profit margins in organic production, the ideal pest management system would naturally sustain biological control agents even when pest populations are low or absent. This would reduce costs while ensuring the grower that the biological controls are there when needed. The marigold guardian plant system does just this. Because this research was conducted in a commercial greenhouse, the grower was directly involved in all aspects of the project. He had input into developing the proposal and took part in research activities such as natural enemy releases and scouting. He and his staff provided insights into evaluating practical aspects of the guardian plant system to ensure it was user-friendly.

A few species of insect-killing fungi have been studied for use against thrips, and preparations based on *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* have significantly reduced thrips populations in greenhouse vegetable and floral crops under research conditions. Fungal products have many

desirable traits—they leave no toxic residues, are generally harmless to beneficials and pose minimal risk to humans and the environment. Unfortunately, foliar fungal applications have provided inconsistent results for thrips control in commercial greenhouses and growers are hesitant to invest in them. The lack of effectiveness may be due to ambient conditions in the foliage which are not ideal for fungal infection, either because of low humidity, solar radiation or too high or low temperature. Efficacy may also be reduced because a portion of the thrips is in the soil, protected from direct contact with the fungal spores. A formulation targeting the soil stage could enhance efficacy. Though insect-killing fungi are commonly formulated as wettable powders, they can be made as a granular, which have advantages over conventional sprays for soil insects. Nutrients can be added into granules to support fungal growth and sporulation. Granules do not need to be incorporated throughout a potting mix but only in the top layer as thrips generally pupate in the upper 2 cm of soil. We developed millet-based granular fungal formulations which, when mixed into growing medium, reduced thrips emergence by over 92% (A. Kassa, unpub. data). In addition, fungal inoculum levels increased over time, providing long-term thrips control in the potting mix and eliminating the need for multiple applications over the season. Control of thrips pupae in potting mix has been reported previously, but it has not been fully tested in U.S. ornamentals.

No biological control agent is a “silver bullet” but must be used as part of a total IPM program for thrips. Early detection is critical so action can be taken before populations reach damaging levels. When spring bedding plants are started in late winter, few thrips may be present on the crop, but populations increase rapidly as temperatures rise. Growers usually rely on yellow sticky cards to monitor thrips. Beneficials are best used as preventatives and introduced during the early stages of an infestation when thrips adults are present in low numbers. Banker plants are used in European and Canadian vegetable greenhouses to provide a continuous supply of beneficials but have not been adopted widely in the U.S. We seek to build on our previous research by combining insect-killing fungi and predatory mites together within a banker/indicator plant system to manage thrips. Through grower-funded research, we found marigolds are highly attractive to thrips and can be used as an indicator plant for early detection (Fig. 1). Because marigolds produce pollen, they also serve as a habitat for predatory mites, providing a food source in the absence of prey. In a related project we tested Hero Yellow marigolds as banker plants, which successfully sustained the predatory mite, *Neoseiulus californicus*, and served as a reservoir for continued predator reproduction. The effectiveness of the banker/indicator plant system could be enhanced by adding a granular formulation of insect-killing fungi in the soil to target pupating thrips. This system would attract thrips from throughout the greenhouse and could protect the crop indirectly.

3. Objectives

1. Determine the effectiveness of marigolds as early pest detection tools and trap plants for thrips in organic greenhouse-grown bedding and vegetable starter plants.
2. Assess the impact on thrips populations of granular insect-killing fungi applied to marigold guardian plants.
3. Evaluate effectiveness of predators and insect-killing fungi within marigold guardian plants to manage thrips in organic greenhouse-grown bedding and vegetable starter plants.

4. Materials and Methods

General Research Setup. Research was conducted in three greenhouses (~3,000 sq. ft) where spring vegetable starter plants were organically grown. Hero Yellow marigolds were produced to the flowering stage in plastic pots (201.1 cm²/pot) from seed at the UVM Entomology Research Laboratory (UVM ERL) in organic potting mix that was sterilized at 121°C for 30 min. This variety of marigold was selected because it is highly attractive to thrips, produces pollen which sustains predatory mites and has a prolific flower type that is suitable as a habitat for mites (Skinner and Frank, unpublished). Marigold plants (<15 cm height) were transplanted prior to resumption of production of vegetable starts. The houses were thoroughly sanitized and weeds removed after the winter fallow period. The presence of resident thrips populations was determined with yellow sticky cards deployed 2 wks prior to placing marigolds in the greenhouses.

One greenhouse, selected randomly, was used to test the marigold guardian plant system “GPS” (GPS greenhouse). Flowering marigolds with a yellow sticky card hung above the plant canopy were placed 2 m apart in the crop (6 per greenhouse) (Fig. 2). We placed the sticky cards in the GPS to enhance their attractiveness to thrips. A granular fungal formulation was prepared using the GHA strain of *Beauveria bassiana*, the fungus contained in BotaniGard® (Emerald BioAgriculture Corp., MT), a commercial spray product used against thrips. A granular formulation of this isolate is not available commercially so we produced it as a millet-based mycelium granule at the UVM ERL. Mycotized millet grains with GHA (1×10^8 viable conidia/g) were incorporated in the upper 2-3 cm of the potting mix at 13.2 g per pot. This rate was used based on earlier lab tests we conducted showing that this was the most effective. Because this trial was done in commercial greenhouses, we relied solely on the natural buildup of thrips. Slow release sachets containing the predatory mite, *N. cucumeris*, were hung on each marigold GPS after the fungal granules were incorporated into the soil. Each sachet was replaced with a fresh one after 6 wks. The number of thrips (adult and larvae) per marigold blossom was determined after 6 and 12 wks. The number of thrips on the surface of soil and that emerged from the soil was counted after the last sampling. Infectivity (mycosis) of the collected thrips from the soil was also investigated.

In another greenhouse (IND greenhouse), flowering marigolds with yellow sticky cards were placed in the same configuration as described above. No granular fungal applications were made to the marigolds but predatory mite sachets were added twice as done for the GPS. The last greenhouse served as an untreated control with only yellow sticky cards (YSC greenhouse) and no marigolds. Data were collected in each greenhouse as described below to address the specific objectives of the project.

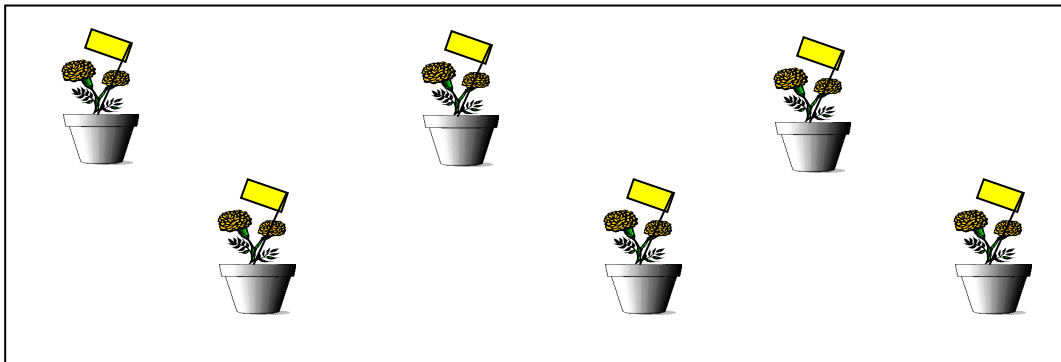


Figure 2: Diagram of trial setup in an organic commercial greenhouse.

Obj. 1. Determine the effectiveness of marigolds as early pest detection tools and trap plants for thrips in organic greenhouse-grown bedding and vegetable starter plants.

We know from previous research that thrips can be detected 1-3 wks earlier on flowering marigolds than on yellow sticky cards or on the crop itself. However, this research had been conducted in commercial greenhouses where standard chemical pesticide-based management was used. It was unknown how this system would perform in a greenhouse where only organic management tactics were used.

Marigold plants in the GPS and IND greenhouses were scouted weekly for 12 wks from early April to early July. Plants were lightly tapped over a white sheet of laminated paper (10 taps/plant) and the number of adult and immature thrips found recorded. Thrips were returned to the plant after counting. In previous research trials we found this sampling method to be reliable for assessing thrips populations on marigolds without disrupting the population dynamics significantly. *N. cucumeris* populations were also assessed weekly by plant tapping and sampling. Flower samples were taken at wk 6 and 12 from the marigold plants to obtain a more reliable assessment of the number of thrips and mites on the plants. Three blossoms from each plant were removed and placed in a ziplock bag with alcohol. They were inspected under high magnification after dissection. A portion

of the crop plants was inspected biweekly for thrips and predatory mites. The greenhouses were divided into six sections. In each section, three plant types were randomly selected and three of each type inspected visually and by tapping over a white sheet of laminated paper (54 plants total per greenhouse) and insects counted.

Overall pest and predatory mite population levels were estimated according to the following rating system: none, very low (≤ 2 /plant/card); low (3-5/plant/card), medium (6-10/plant/card), high (11-20/plant/card), very high (>20 /plant/card). Yellow sticky cards were inspected, and the number of thrips recorded. In the YSC greenhouse, only crop inspections and yellow sticky card counts were made.

Obj. 2. Assess the impact on thrips populations of granular insect-killing fungi applied to marigold guardian plants.

The use of a granular formulation of insect-killing fungi represents a new approach to microbial biological control. Therefore, data was to be generated to specifically evaluate this treatment. To assess fungal infection of thrips, after 12 wks thrips were collected from plants and hand-picked from the soil surface. They were surface-sterilized using the method of Shipp et al. (2003). They were then placed in Petri dishes with selective medium for *B. bassiana* (Doberski and Tribe 1980; Beilharz et al. 1982) and held at $25 \pm 1^\circ\text{C}$ for 5-7 d. The number of infected thrips was counted based on growth of mycelium and the morphology of produced conidia.

To assess the emergence of thrips from soil, plants were clipped at the soil surface and discarded. Each pot was then covered with a clear sticky lid, sticky side down, ensuring that soil did not touch the sticky surface of the lid. The sticky lids were made with clear plastic sheets coated with a thin layer of TanglefootTM (Tanglefoot Co., Grand Rapids, MI). The sticky lids were secured with rubber bands. Pots were kept at room temperature for 10 d, away from direct sunlight and heat. The number of thrips per sticky lid was counted using a magnifying glass (10 \times). Thrips on the lids were detached aseptically and held on the selective medium to determine fungal infection using the method described above.

The level of *B. bassiana* fungal colonization in soil was determined at 12 wks after the transplanting. The soil in each pot was mixed in a plastic box. A 20 g subsample was taken and mixed with 0.02% polysiloxane polyether (Silwet L-77R) solution to make a 50-ml soil suspension. An additional 5 g was taken to measure dry weight based on the gravimetric water content (Lambe and Whitman 1969). These data were used to express the fungal population based on dried potting soil. Diluted soil suspensions (100 μl) were spread on SDAY medium and held at $25 \pm 1^\circ\text{C}$ for 3-5 d to determine the number of colony forming units (cfu).

Obj. 3. Evaluate effectiveness of predators and insect-killing fungi within marigold guardian plants to manage thrips and other arthropod pests in organic greenhouse-grown bedding and vegetable starter plants.

It would be ideal to evaluate each of the biological control components (predatory mites and fungi) separately and together within the same greenhouse to determine individual and combined impacts. However, because thrips and their predators are so mobile, we find it difficult to effectively test different treatments within one greenhouse. Therefore, we conducted the research in three different greenhouses, comparing the effect of using the GPS with the fungi with marigolds and mites alone or no marigolds at all. In other research, we have conducted caged trials under laboratory conditions to determine the effect of individual components on thrips populations. Using data from the guardian plants, yellow sticky cards and randomly selected crop plants (RPI) on the number of thrips and predatory mites, we could determine the effectiveness of the guardian plants.

5. Project Results

Thrips Infestation Levels on Sticky Cards, Marigolds and Randomly Inspected Plants. Low levels of thrips were established in the GPS and IND greenhouses up to 4 wks before the treatments were added. More thrips were found on yellow sticky cards in the GPS and IND greenhouses than in the control (YSC) house. The mean number of thrips per card averaged over the entire test period was 5.71, 6.40 and 1.15 thrips per card in the GPS, IND and control houses, respectively. These numbers suggest a moderate thrips infestation in the two

houses with marigold systems and a very low infestation level in the untreated control. By wk 12, fairly high numbers of thrips per card (~15-25/card) were observed in the GPS and IND house (Fig. 3A). No significant statistical differences were found in the number of thrips on sticky cards in the two treatment greenhouses GPS and IND.

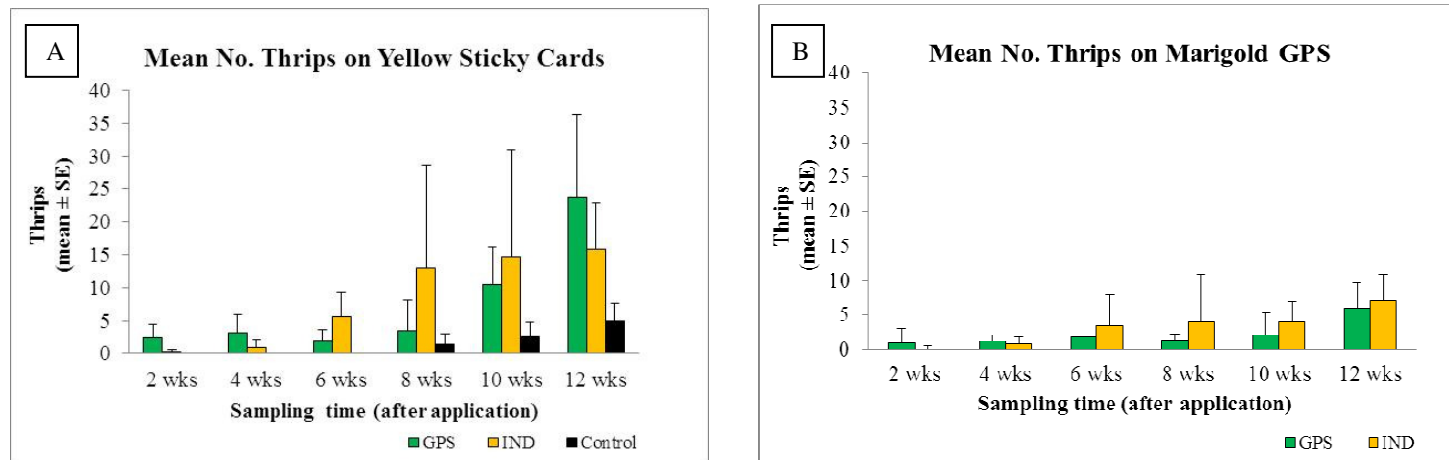


Fig. 3. A. Thrips on yellow sticky cards in the three test houses (GPS=Guardian Plant System: marigolds with fungus and mites; IND=marigolds with mites; Control=marigolds with yellow sticky cards); B. Thrips on marigolds in the GPS and IND houses.

In the GPS and IND treatment, greenhouse thrips populations on the marigolds ranged from none to high (11-20/plant) (Fig. 3B). When averaged over the entire test period, there were slightly less thrips/marigold in the GPS house (2.25/marigold) than the IND house (3.28/marigold), though differences were not statistically significant. At the first sampling period, thrips numbers were 1.0 ± 2.0 thrips/marigold (GPS) and 0.2 ± 0.4 thrips/marigold (IND) while by the end of the 12-wk test period, thrips had increased only slightly, to 6.0 ± 3.9 (GPS) and 7.2 ± 3.8 thrips/marigold (IND) (Fig. 3B). Little to no damage (<10% foliar damage) was observed on the marigold plants in both treatments with no significant difference between them. On randomly inspected plants, biweekly thrips infestation levels ranged from none to low (3-5/plant).

Predatory Mite and Thrips Populations on Marigold GPS. Using the plant tapping sample method, very low numbers of mites were found on the marigolds and none were found on randomly inspected crop plants (Fig. 4). In contrast, some of the blossom samples from these same marigolds had very high numbers of predatory mites and thrips per blossom (>20/blossom). This indicates that the plant tapping method does not accurately reflect mite populations on the plant. As in year 1 of this project, our data suggest mites tended to remain in the marigold blossoms and did not readily disperse into the crop. After the first sampling at 6 wks, there were 0.2 - 2.2 mites/marigold in the GPS greenhouse compared with 0.3 - 2.0 mites/marigold in the IND house. Similar levels of mites were found after 12 wks and differences in the number of mites in each treatment were not significantly different (Fig. 5B). Fewer thrips were found within flowers in the GPS than the IND greenhouse at 12 wks (Fig. 5A). This suggests that, over time, thrips populations are suppressed more effectively within a marigold on which predatory mites and a fungal treatment is applied than one with mites only.

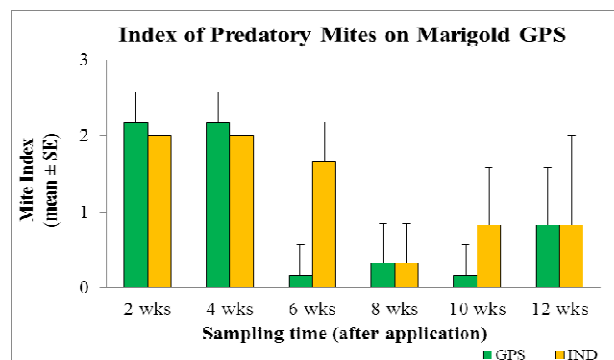


Fig. 4: Index of predatory mites on marigolds in test greenhouses.

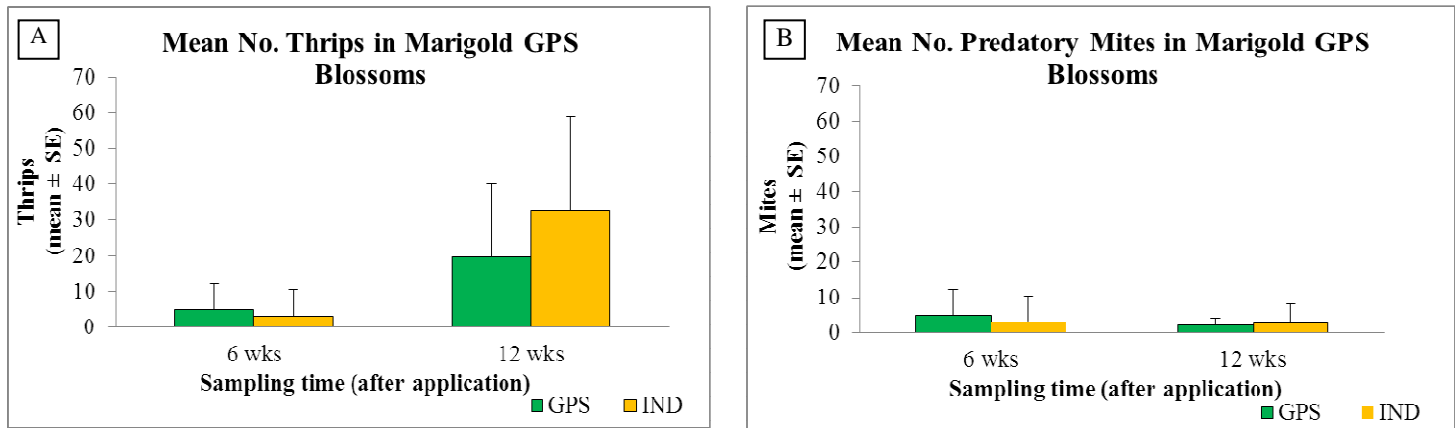


Fig. 5. Mean number of thrips (A) and predatory mites (B) in marigold blossoms in the two test houses (GPS=Guardian Plant System: marigolds with fungus and mites; IND=marigolds with mites).

Fungal Treatment Effect and Persistence. We observed significant fungal outgrowth from the granules on the potting soil surface throughout the experiment and evidence of fungal infection of thrips was observed in samples from the GPS treatment. At 12 wks, an average of 3.3 ± 1.9 thrips/plant were collected from marigold leaves in the GPS treatment, similar to the number for the IND treatment (4.7 ± 2.7 thrips/pot). Of those thrips collected from the GPS treatment, $65.0 \pm 20.4\%$ were infected with the fungus, whereas none of the thrips from the IND treatment were infected. Thrips that were still alive on the marigolds in the GPS greenhouse probably would have died within a few days from infection or predation by mites. Very low numbers of thrips (< 1 /pot) were observed on the surface of soil in both treatments. All of the thrips from the GPS treatment were infected while none of the thrips from the IND treatment were. On the surface of the soil of the two treatments, similar numbers of predatory mites were observed (30.0 ± 15.2 /pot for the GPS treatment; 46.7 ± 13.7 /pot for the IND treatment). Similar numbers of thrips were caught on the sticky lids for both treatments (< 2 thrips/pot) and none were infected with the fungus. Predatory mites on the soil surface probably controlled thrips that fell down from the plants, which could explain the low levels of thrips emergence as measured by numbers on the lids.

6. Conclusions and Discussion

Overall, the marigold GPS that combined a granular fungal treatment and predatory mites appeared to suppress thrips more effectively than marigolds with predatory mites alone. The specific role of *B. bassiana* GHA millet grains, however, still needs further evaluation to confirm its efficacy. This research was conducted in commercial greenhouses. As a result we relied on a natural build-up of thrips. Overall thrips infestation levels in the test greenhouses were fairly low to medium. Thrips were present, but not in numbers substantial enough to cause serious damage. This was great for our cooperating grower, but didn't provide an opportunity to assess the GPS under conditions of high pest pressure. Below are responses to practical questions from the trial.

Are marigolds more attractive to thrips than bedding plants and an effective tool for their early detection? Thrips were found faster and in higher numbers on sticky cards and marigolds than on the crop plants. At any given observation point, more thrips were found on individual marigold plants and cards than on any one randomly inspected plant. Our past research has shown that to enhance attractiveness, marigolds should be in flower and ideally placed in the greenhouse well before the crop begins flowering. For these trials, marigolds were often the only flowering plant in the greenhouses as most of the crops were vegetable starter plants. Thrips damage on the crop was minimal and very patchy when it occurred. Very few thrips were found on the crops in the test greenhouses, suggesting they were attracted to flowering marigolds and sticky cards rather than the crop, and demonstrating the suitability of marigolds as a trap crop and early detection tool for thrips.

Are the predatory mite populations sustained on guardian plants, and do they reproduce there? Predatory mites were present on the marigolds throughout the entire experiment. They were present in higher

numbers in the blossoms than on the foliage as in year 1. We know that the predatory mites, when applied using a slow release sachet, can persist for at least 6 wks. There were substantial numbers of mites in the blossoms over time suggesting that they readily colonize the blossoms, which appear to provide a suitable habitat. In the first year, we used inundative releases of mites. By using sachets, growers could save a significant amount of money from reduced shipping costs while obtaining a similar level of thrips management.

Do thrips become infected following application of a granular fungal formulation on potting soil?

In the GPS treatment 12 wks after the trial began, all of the thrips collected from the soil surface and 65% of those collected from the foliage were infected. None of the thrips from the IND treatment on the soil surface or on foliage showed signs of infection. This suggests that thrips definitely become infected from contact with the granular formulation. Because so few thrips were captured following emergence, it was impossible to determine when infection occurred. The apparent low emergence could be explained by the fact that thrips which enter the soil to pupate do not survive to emerge, or that predatory mites consume them either after they fall to the ground or before they reach the sticky lid. More research should be conducted on the process and timing of thrips infection to fully understand how the GPS system works.

Are guardian plants (with fungi and mites) effective for reducing thrips in spring bedding plants?

Though thrips populations were low and no significant differences were found between the two treatment greenhouses, overall lower numbers of thrips were found on marigolds in the GPS than the IND greenhouse. This suggests that the biological control agents may have been affecting thrips populations on the GPS. Because we tested the predatory mite separately, we can speculate that the lower thrips populations in the GPS treatment were a result of the fungus. More greenhouse trials must be conducted to fully assess the potential of this system.

How long does the fungus remain viable and infective following application? Evidence of sporulating fungal granules was observed throughout the duration of the experiment. In year 1, we were concerned that the fungal inoculum would leach out of the potting mix as a result of regular overhead watering. Therefore, we conducted and published a study to assess fungal spore concentrations in the water that escapes from the bottom of flower pots during watering. Spore concentration levels in the residual water were determined over an 18-day period after the fungal granules were applied. We found that the concentration of *B. bassiana* was greatest in the upper layer of the potting medium, and no significant movement downward was detected (Kim et al. 2010).

7. Outreach

Conducting research in commercial greenhouses in collaboration with growers is an ideal opportunity for highly effective and targeted outreach. The grower with whom we cooperated now uses marigolds in all of his greenhouses as a trap crop based on his observations of the success of our research. He has also encouraged other growers to follow his example. Though one-on-one training is ideal, it is generally cost-prohibitive. We have been holding hands-on IPM workshops in Maine, New Hampshire and Vermont for greenhouse growers for over 14 years. Results from this project were disseminated to growers at these workshops in January 2011. Over the past 2 years, 22-53% of growers who attended the workshops indicated they used some form of plant-mediated IPM system in the past year. Marigolds were the most commonly used plant used for this purpose.

8. References

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9. Addenda

Below is a gallery of pictures of the project.



Marigold GPS early stage crop production with mite sachet and yellow sticky card.



Millet-based fungal granules sporulating (red arrow) on the soil surface beneath a marigold GPS.



Marigold GPS with mite sachet (red arrow) and sticky card in greenhouse crop of vegetable starter plants.



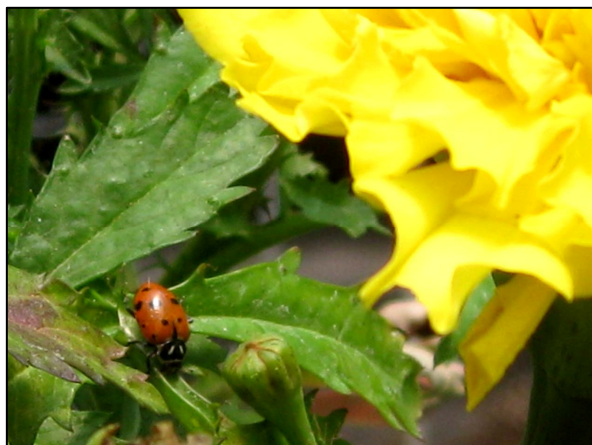
Thrips adults on marigold GPS blossom.



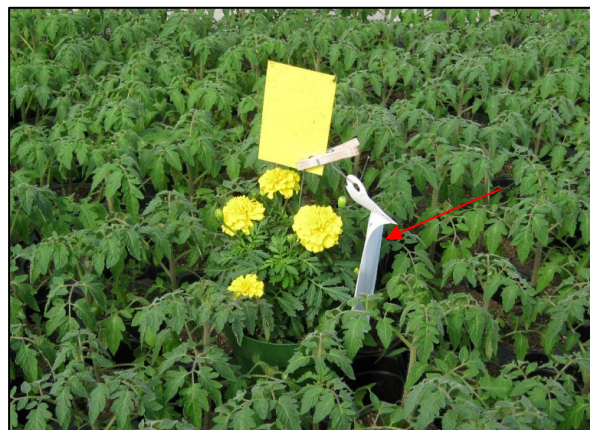
Thrips damage on foliage of marigold GPS. The crop plants escaped major damage.



Yellow sticky cards in control greenhouse.



Naturally-occurring lady beetle predator which was frequently observed visited marigold GPS and likely contributed to reducing thrips populations on foliage.



Marigold GPS with yellow sticky card and mite sachet (red arrow) deployed in pre-finished starter tomatoes.