ORGANIC FARMING RESEARCH FOUNDATION

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This is a final project report submitted to the Organic Farming Research Foundation.

Project title:
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 western flower thrips (WFT) in a commercial greenhouse of organically-grown spring bedding plants, combining predatory mites, granular insect-killing fungi and marigolds into one effective "guardian plant" system (GPS). The assumption was that adult thrips would be attracted out of the crop to the flowering marigolds, where they would become established. The immature thrips would serve as prey for the predatory mite, *Neoseiulus cucumeris*, sustaining them and encouraging their dispersal through the crop. Thrips escaping predation would drop to the soil to pupate, where they would become infected with fungi. The granular formulation would enable the fungus to colonize the potting mix, eliminating the need for reapplication. This represents a low-cost, organic approach, achieving WFT 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 will be reduced, while control is maximized.

Marigolds were tested as a GPS in greenhouses of organic bedding crops: one with the GPS and predatory mites and fungi, one using marigolds as trap plants with no biological controls and one with no marigolds. Predatory mites were released in the GPS canopy every other week and the soil was treated with granular fungi once before placing then in the greenhouse. For 13 weeks thrips and mite numbers were monitored weekly with plant tapping and blossom sampling on marigolds and a random assortment of crops and yellow sticky cards. Low levels of thrips were present throughout the growing season in all greenhouses. More mites were present in the marigold flowers than on foliage. Thrips were also found in the blossoms. When thrips populations were compared among treatments, marigold flowers from plants treated with biological controls had fewer thrips than those without treatment. Thrips numbers were also lower on sticky traps in the biocontrol greenhouse than the other houses. Because thrips numbers were low in both the control and treatment greenhouses, conclusions about the effectiveness of the treatments cannot be definitively drawn. However, the study did demonstrate that overhead watering does not leach the fungal inoculant out of the soil. Collectively, these results suggest that the marigold GPS has potential as a useful tool for thrips management in organic greenhouses. This work needs to be repeated to confirm the conclusions.

2. Introduction

This project addressed a serious pest of organic greenhouse production nationally. In Vermont, thrips are the most common reason for organic growers suspending organic practices 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 2 years supported by the USDA and national grower associations to assess marigolds as indicator plants. We found marigolds were highly attractive to WFT 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 WFT 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 2 months as they do over an entire summer of selling vegetables (J. Mannix, 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. 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 WFT 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 the needs of organic growers, the results will also benefit non-organic growers who seek to achieve more ecological pest management.

Fig. 1. Indicator plant in commercial greenhouse (grower-funded research).

Current recommended biological control strategies for this pest, which commonly require weekly releases of natural enemies, 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 the research was conducted in a commercial greenhouse, the grower was directly involved in all aspects of the project. They had input into developing the proposal and took part in research activities such as natural enemy releases and scouting. They 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 WFT, and preparations based on *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* have significantly reduced WFT 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 WFT 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 WFT 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 WFT generally pupate in the upper 2 cm of soil. We developed millet-based granular fungal formulations which, when mixed into growing medium, reduced WFT emergence by over 92% (A. Kassa, unpub. data). In addition, fungal inoculum levels increased over time, providing long-term WFT control in the potting mix and eliminating the need for multiple applications per season. Control of WFT pupae in potting mix has been reported 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 WFT. Early detection is critical so action can be taken before populations reach damaging levels. When spring bedding plants are started in late winter, few WFT may be present on the crop, but populations increase rapidly as temperatures rise. Growers usually rely on yellow sticky cards to monitor WFT. Beneficials are best used as preventatives and introduced during the early stages of an infestation when WFT 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 WFT. Through grower-funded research, we found marigolds are highly attractive to WFT 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 for management of spider mite. We found that this variety of marigold successfully sustained the predatory mite, *Neoseiulus californicus*, and served as a reservoir for continued predator reproduction. We believed the effectiveness of the thrips banker/indicator plant system could be enhanced by adding a granular formulation of insect-killing fungi in the soil to target pupating WFT and using *N. cucumeris*, a thrips predator. This system would attract WFT 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 greenhouse bedding 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 and other arthropod pests in organic greenhouse-grown bedding plants.

4. Materials and Methods

General Research Setup. Research was conducted in three greenhouses (~3,200 sq. ft) where spring bedding, foliage and vegetable starter plants were organically grown. The greenhouses are certified by Vermont Organic Farmers. 'Hero Yellow' marigolds were produced to the flowering stage from seed at the UVM Entomology Research Laboratory (UVMERL).

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 & Frank, unpublished). Prior to resumption of production, the houses were thoroughly sanitized using OxiDate® and weeds removed after the winter fallow period. Resident thrips populations were assessed with yellow sticky cards 1 week prior to placing the marigolds in the greenhouses.

One greenhouse, selected randomly, was used to test the marigold guardian plant system 'GPS' (GPS treatment greenhouse). Flowering marigolds were placed at least 2 m apart in the spring bedding plant crop (6 per greenhouse) (Fig. 2). A granular fungal formulation was prepared using the GHA strain of *Beauveria bassiana* (the active ingredient in many

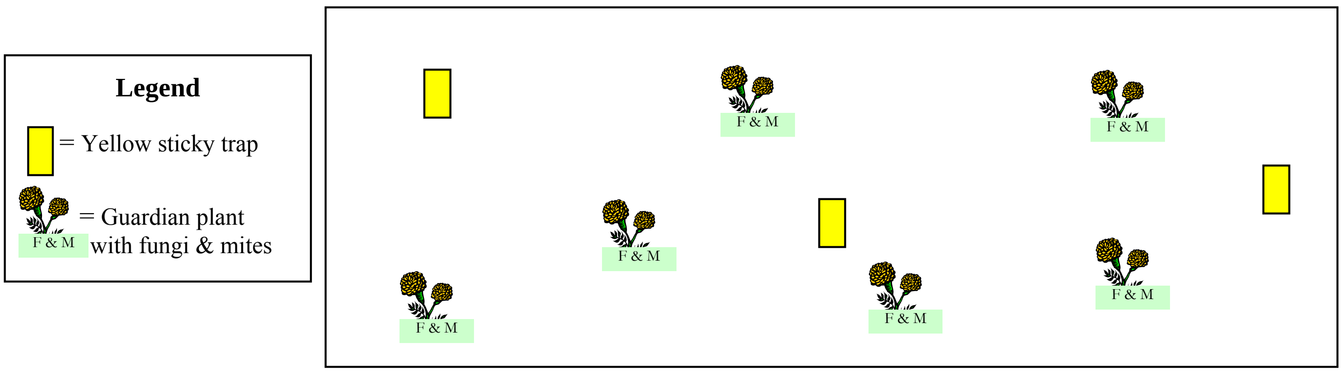


Fig. 2. Diagram of trial setup in an organic commercial greenhouse.

mycoinsecticides). Since a granular formulation of this isolate is not available commercially, we produced it as a millet-based mycelium granule at the UVM ERL. (See appendix for a description of the production process.) The granular fungal material was applied to the GPS at a rate of 6 g/pot (1 g granules ≈1.2 x 10^8 conidia). Granular treatments were applied once to the potting mix by sprinkling it around the base of the marigold. We are currently testing the efficacy of different application rates under controlled laboratory conditions. However, for this project only one rate was tested. In future trials, other application rates could be tested based on the results of our laboratory research. Because this trial was done in commercial greenhouses, we relied solely on the natural buildup of thrips. Inundative releases of the predatory mite *N. cucumeris* were made on guardian plant foliage every 2 weeks at a rate of 2 tablespoons (~ +/- 850 mites) per plant. Yellow sticky cards were placed at a rate of 1 per 1,000 sq. ft. according to standard guidelines.

In another greenhouse (IND greenhouse), flowering marigolds and yellow sticky cards were placed in the house at the same configuration as in the GPS greenhouse, described above. No granular fungal applications or predatory mites were made to the marigolds. The last greenhouse served as an untreated control with only yellow sticky cards (YSC greenhouse) and no marigolds. It primarily contained vegetable starter plants. Data were collected in each greenhouse as described below to address the specific objectives of the project.

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

We know from previous research that WFT 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 is unknown how this system will perform in a greenhouse where only organic pest management is used.

Marigold plants in the GPS and IND greenhouses were scouted weekly for 13 weeks 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 and dissecting of three flowers per plant. A portion of the crop plants was inspected for thrips, predatory mites and other arthropods. The greenhouses were divided into six sections. In each section 3 plant types were randomly selected and 3 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); low (3-5/plant), medium (6-10/plant), high (11-20/plant), very high (>20/plant). Yellow sticky cards were inspected, and the number of thrips and other arthropods (e.g., aphids, whiteflies, fungus gnats, shoreflies) recorded. In the YSC greenhouse, only crop inspections and yellow sticky card counts were made. Scouting times for each variable and the time it took to find the first thrips were also recorded.

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

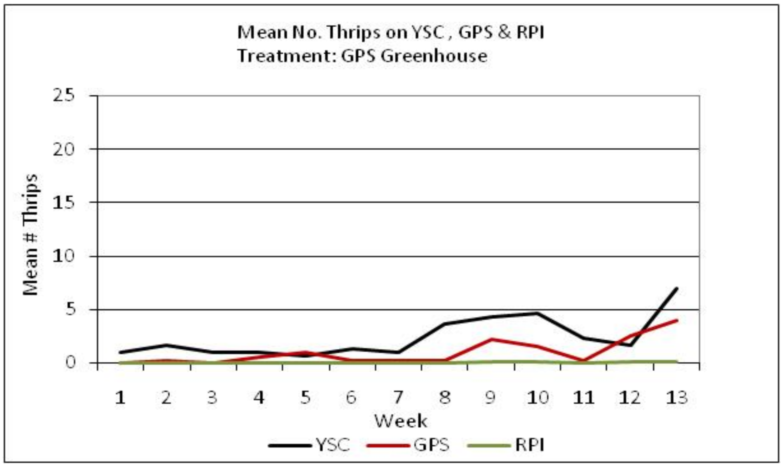
The use of a granular formulation of insect-killing fungi represents a new approach to microbial biological control. Samples of infected thrips were taken from the foliage and potting mix of the marigolds, surface-sterilized, placed in a moist chamber and incubated at 25°C for 10 d. The presence/absence of *B. bassiana* infection was determined. Samples (~1 g) of the potting mix were taken to assess fungal colonization.

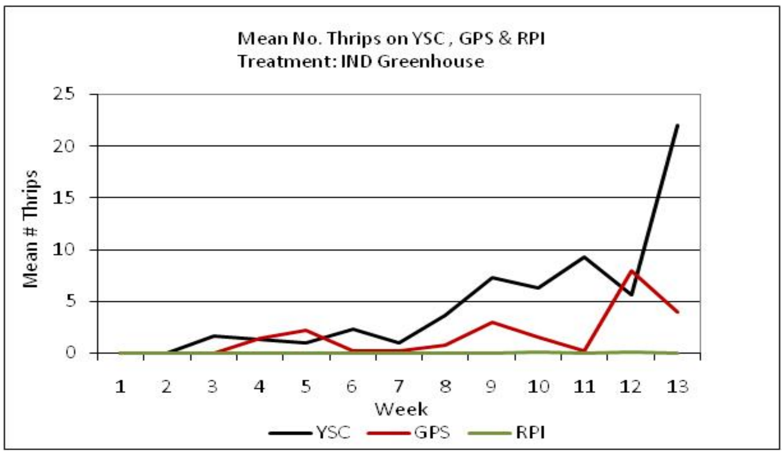
Obj. 3. Evaluate effectiveness of predators and insect-killing fungi within marigold guardian plants to manage thrips and other arthropod pests in organic greenhouse-grown bedding plants.

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

5. Project Results

Thrips Infestation Levels on Sticky Cards, Marigolds and Randomly Inspected Plants. In all of the greenhouses, thrips population levels were very low (≤2 thrips/plant) to low (3-5 thrips/plant) with little noticeable damage (<10% per plant). In the GPS treatment greenhouse very low levels of thrips were recorded throughout the season on marigolds (mean=1.07), yellow sticky cards (mean=2.41) and randomly inspected crop plants (mean=0.05). In the greenhouse with marigolds without biological control agents (IND greenhouse), very low levels of thrips were recorded on marigolds (mean=1.8) and randomly inspected crops (mean=0.04) and low levels detected on yellow sticky cards (mean=4.74). In the control greenhouse (YSC) with yellow sticky cards only, very low levels of thrips were recorded on yellow sticky cards (mean=1.58) and randomly inspected plants (mean=0.02). The IND greenhouse has slightly higher numbers of thrips over time than the GPS greenhouse (Fig. 3). It should be noted that because of the virus threat, many conventional growers would apply a chemical pesticide if as few as 2 thrips per yellow sticky card was found.





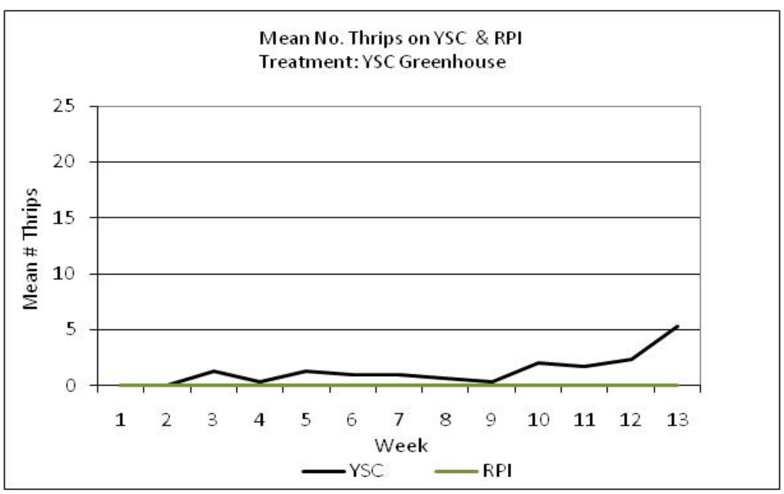


Fig. 3. Thrips population trends in the three test greenhouses (GPS with the treated marigolds, IND with untreated marigolds and YSC with no marigold treatment). YSC=yellow sticky card, GPS=guardian plant system, RPI=random plant inspections. Note: the greenhouse with YSC only contained non-flowering vegetable starter plants, and thus less attractive to thrips. Therefore thrips data from that greenhouse were not directly comparable with the other houses.

In all greenhouses more thrips were found on the marigolds and sticky cards than on the randomly inspected plants. Average scouting times were as follows: sticky cards (5 minutes), marigolds (10 minutes) and random plant inspections (15 minutes). On average when inspecting marigolds, it took 2 minutes to find the first thrips compared to an average of 4 minutes on the randomly inspected crops. This suggests that sticky cards and marigolds are effective monitoring tools that save growers time by detecting thrips quickly.

Predatory Mite Populations on Marigold GPS. Very low numbers of mites (mean=1.11) were observed from tapping the GPS. No predatory mites were found on randomly inspected plants. Blossom samples from the same marigolds showed very high numbers of predatory mites per blossom (mean= 22.23). These data suggest mites preferred to remain in the marigold blossoms rather than dispersing in the crop.

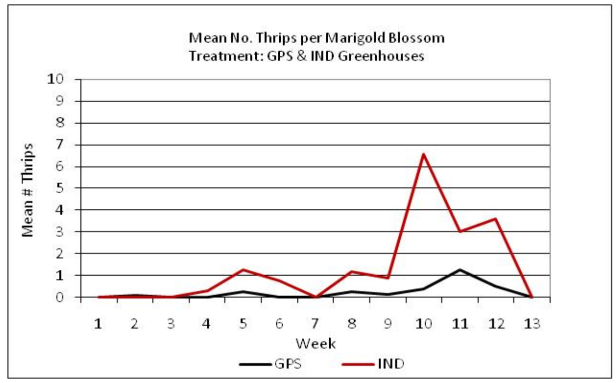


Fig. 4. Mean number of thrips from blossoms of treated and untreated marigolds.

More thrips were found within flowers in the IND (mean=1.76) than the GPS greenhouse (mean=0.29) (Fig. 4). These data suggest that marigolds with predatory mites and fungi have lower thrips populations in the blossoms than marigolds that have no biological control agents. This indicates that marigolds combined with predatory mite releases and fungi provide a useful thrips management tool.

Fungal Treatment Effect and Persistence. We were not able to check thrips cadavers for fungal infection because no infected thrips were observed on the foliage or in the flowers. This may be due to the fact that any thrips larvae or pupae that became infected in the soil died there and did not emerge as an adult to feed on the plant. We observed significant fungal outgrowth from the granules throughout the course of the experiment. This eliminated the need to complete extensive fungal load studies.

6. Conclusions and Discussion

This research was conducted in commercial greenhouses. As a result we relied on a natural buildup of thrips. In 2008, thrips populations were unusually low for most Vermont growers, perhaps because of the cool, rainy and cool conditions throughout the season. Thrips were present, but not in numbers substantial enough to cause serious damage. Low levels of thrips were present in all test greenhouses throughout this trial. The following are responses to our practical questions from these trials.

Are marigolds more attractive to thrips than bedding plants? Thrips were found in higher numbers on the marigolds than on the bedding plants. An individual marigold plant would often have higher numbers of thrips at a given observation point than any one randomly inspected plant. Our previous research has shows that it is important that the marigolds be flowering early well before the crop to enhance its attractiveness. Marigolds were placed in this greenhouse in early April after many of the crops were established. Some plants in the greenhouse already began flowering by this time providing another attractant to thrips. The marigolds used here began to flower late and their initial quality was fair for the first few weeks. After the marigolds were placed in the greenhouses from the lab they stressed from a freak early heat wave as well as from stress from sitting in saucers full of water (i.e., overwatering) setting their growth back and affecting their quality. After a few weeks the plants fully recovered from the initial stress of moving from one environment to another and began to flower again. Thrips were also already present in these greenhouses at the start of the experiment before the marigolds were placed in the greenhouses. Marigolds are potentially very useful for attracting thrips and drawing them out of the crop, but only if they are placed in the crop early, one of the only plants flowering and if thrips have not already established themselves in the other crops.

Are marigolds an effective scouting and early detection tool? It took less time to scout marigolds and sticky cards than random plant inspections. Thrips were also detected much faster on the marigolds than by searching for them on randomly selected crop plants. Thrips were detected at the same time on the marigolds as on the sticky cards. In fact thrips were found in the greenhouses before the experiment started to the surprise of the grower. Considering how much faster thrips were found on marigolds than on the crop, we feel growers could save time and money by using marigolds as an indicator plant for early detection.

Are the predatory mite populations sustained on guardian plants, and do they reproduce there? After the initial release, predatory mites were present throughout the season on the marigold GPS. They were present in higher numbers in the blossoms than on the foliage. For this trial we used routine inundative releases of mites rather than making one release and waiting to see how long they persisted. 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. Further research on the persistence of mites over time is needed to determine how often mites should be released onto the GPS. Reducing the rate of release would save growers significant money.

Do WFT become infected following application of a granular fungal formulation on potting soil? We could not address this question because no infected thrips were observed on the foliage.

Are guardian plants (with fungi and mites) effective for reducing WFT in spring bedding plants? Though thrips populations were low, we did observe consistently lower numbers of thrips in the GPS than the IND house, suggesting the biological control agents were affecting thrips populations on the GPS and the crop overall. Because we were unable to test each biological control agent separately, we can't determine if the lower populations were a result of the mites, the fungus or both. However, our results did demonstrate that the GPS combining mites and fungi reduced populations.

How long does the fungus remain viable and infective following application? Evidence of sporulating fungal granules was observed throughout the duration of the experiment. Because we were concerned that the fungal inoculum would leach out of the potting mix as a result of regular watering, saucers to hold the water were placed under each of the marigold pots which received granular fungal treatments. We asked the grower to water these plants from the bottom. Within a few weeks we noticed a bad smell coming from the marigold plants and after further investigation determined it was coming from the water in the saucers. We also noticed that the plants were routinely being watered from above despite our instructions. It became clear that it was unrealistic to expect growers to water the marigold GPS differently from the other crop plants. Therefore we conducted a preliminary trial 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. Over 80% of the spores were found in the top 1/3rd of the pot, and few spores were found in the water that drained out of the pot. Therefore we believe top watering does not negatively affect the fungal treatment.

7. Outreach

Conducting research in commercial greenhouses in collaboration with growers is an ideal opportunity for highly effective and targeted outreach. For example, when we initiated contact with our cooperating growers they were happy to have us do the research in their greenhouses, but they warned us that they had never had western flower thrips or thrips of any kind. On the day we brought the marigold plants, we inspected the crop to determine baseline thrips levels. The grower insisted there were no thrips, but within 5 minutes we had found thrips on yellow sticky cards and crop plants. This was indeed a learning experience for the grower. Though they have attended workshops on pest identification for many years, until there was someone in their greenhouse pointing out the pest, they hadn't transferred the knowledge to their own operation.

Though one-on-one training is the ideal, it is generally cost-prohibitive. We have been holding hands-on IPM workshops in Maine, New Hampshire and Vermont for greenhouse growers every January for over 10 years. Results from this project were disseminated to growers at the annual tri-state Greenhouse IPM workshops held in Maine, New Hampshire and Vermont in January 2009. Based on information collected through exit evaluations from workshop participants, 60% of growers who attended last year's workshop said they used a new tactic learned at the workshop, most commonly plant-mediated IPM systems such as habitat plants. Over 41% of the growers indicated they use some form of a plant-mediated IPM system last year, compared with 39% the year before. This IPM tool seems to be particularly interesting to growers and something they are readily willing to implement. Work is underway to prepare an article on the research for submission to a national trade journal. A poster was designed in cooperation with our grower partner describing the study and its benefits for putting up in retail space to inform the public of the work. Posters were put up in retail spaces frequented by the public. Customer satisfaction is key to marketing, and our previous surveys suggest that the public prefers to purchase plants grown with as little chemical inputs as possible, as long as the quality of the plant is not reduced. Thus public education is as important as disseminating the results to growers.

8. Appendix

Methods for Mass Production of a Granular Formulation of Insect-Killing Fungi for a Habitat Plant System Tested with Funds from the Organic Farming Research Foundation

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I. Two-stage liquid culture process

The fungal isolate used for this study was GHA, the *Beauveria bassiana* isolate found in BotaniGard® (Emerald BioAgriculture Corp., MT), a registered commercially-available fungal-based spray product. A single-spore isolate of the test entomopathogenic fungus (GHA) was stored in 10% (v/v) glycerol at -80°C. After removal from cold storage, it was grown up in a Petri plate on potato dextrose agar (PDA, Difco) at 24 ± 2°C for 14 d (Humber 1997). Fungal conidia, which served as inoculum for the liquid culture, were harvested from the plate and a 1-ml conidial suspension (1×10^7 conidia ml^{-1}) was inoculated into the flask. A liquid culture to provide inoculum for mass production on the solid substrate (see below) was produced in 100 ml of liquid PDA in a 250-ml baffled flask held at 24 ± 2°C for 3 d (Jenkins et al. 1998).

II. Solid culture process

Millet (*Panicum miliaceum*) grains (500 g; from local organic health food store), were placed in a polyvinyl bag (60×30 cm^2), and soaked in 250 ml of water containing citric acid (0.4 ml l^{-1}) at 90-95°C for 2 h as a preliminary cooking step (Li and Feng 2005). The grains were autoclaved at 121°C for 60 min to sterilize the media completely and cooled to ambient temperature. The bag was flattened by wrapping it around a 4-cm diameter test tube without overlapping and folding. The bag was sealed loosely with a rubber band during cooling. The liquid broth (5.0 ml), prepared in the liquid culture (see above), was introduced into the bag. The bag was shaken for approximately 1 min to distribute the inoculum evenly over the grains. Finally, the bag was sealed with a sterilized paper sleeve, covered with three layers of sterile cheese cloth, to allow for air exchange without contamination. It was incubated at 24 ± 2°C [L:D 12:12] for 3 wk.

III. Drying of mycotized millet grains

After the 3-wk of incubation, the bag was opened and allowed to dry at ambient temperature for 10 days. Alternatively, the open bag could be opened and allowed to dry for 2 days in a small drying room with a dehumidifier. The material was considered ready for application when the moisture content was less than 5%.

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