

## PROJECT SUMMARY

**Instructions:**

The summary is limited to 250 words. The names and affiliated organizations of all Project Directors/Principal Investigators (PD/PI) should be listed in addition to the title of the project. The summary should be a self-contained, specific description of the activity to be undertaken and should focus on: overall project goal(s) and supporting objectives; plans to accomplish project goal(s); and relevance of the project to the goals of the program. The importance of a concise, informative Project Summary cannot be overemphasized.

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**Title:** Fungi, Predatory Mites And Habitat Plants For Thrips Ipm In Greenhouse Ornamentals

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This is a Research project testing a novel approach for IPM of western flower thrips (WFT) in spring bedding plants, combining predatory mites, granular entomopathogenic fungi and indicator plants into one effective “habitat plant system”. Adult WFT will be attracted from the crop to flowering marigolds, where they will become established. A portion of the immature WFT will serve as prey for the predatory mite, *Neoseiulus cucumeris*, sustaining the mite population, and encouraging dispersal throughout the greenhouse. A portion of the WFT escaping predation will drop to the soil to pupate, where they will be targeted with fungi. The granular formulation will enable the fungus to colonize the potting mix, eliminating the need for repeat applications. This concept represents a sustainable, low-cost, ecological approach to combating the most serious pest facing greenhouse growers today. This project addresses all three RIPM program goals. It will contribute to safeguarding human health and the environment by reducing growers’ use of chemical insecticides, thereby lessening exposure of applicators, growers, agricultural workers and the public to toxic compounds. This study tests an innovative IPM strategy that could offer economic benefits to growers by reducing WFT damage, increasing plant quality, and minimizing production costs by providing a sustained source of biological control agents within the crop. This could lead to wider adoption of IPM by growers, thousands of whom in the Northeast struggle annually with WFT control. Growers would surely adopt this IPM strategy if they knew it worked better and cost less than chemical control.

**Fungi, Predatory Mites and Habitat Plants for  
Thrips IPM in Greenhouse Ornamentals**

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## PROJECT NARRATIVE

### i. Problem, Background and Justification

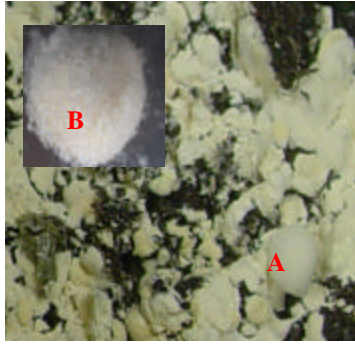
**The Problem.** Western flower thrips (WFT) [*Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae)] remains one of the most important pests of protected agriculture in the US and world-wide<sup>1,2</sup>. They are identified by growers as a primary pest throughout New England, where in some crops, pesticides are applied every 3-7 d<sup>3</sup>. WFT feeds on a wide range of greenhouse ornamentals, causing cosmetic damage that reduces crop value and transmitting virus diseases, which can destroy an entire crop if WFT is not controlled<sup>4</sup>. Its cryptic behavior, rapid reproductive rate and potential to develop resistance to insecticides make WFT particularly difficult to control<sup>5,6</sup>. Several predatory mite species are used to combat WFT in the aboveground parts of the plant<sup>7,8,9</sup>. However, a large portion of WFT populations pupate in the soil, which is generally overlooked as an environment to target for management<sup>10,11</sup>. Formulations of entomopathogenic fungi are available for foliar application against WFT but are not widely used and reports of their efficacy have been inconsistent. Granular fungal formulations show promise in laboratory trials but are not currently available commercially.

In the US, about 25,000 farming operations produce nursery and greenhouse crops with a total annual wholesale value of \$14 billion<sup>32</sup>. The income-generating potential of greenhouse ornamentals far exceeds that of most traditional crops in New England. Based on the most recent USDA NASS survey of 6 of the 13 New England states, the annual wholesale value in 2004-05 exceeded \$460 million, an increase of 17.5% since 2000<sup>30,31</sup>. Though only 2,000 of the operations produce 65% of the revenue for this crop nationally, most of the producers manage comparatively small operations, which is why this industry is critical to the expansion and sustainability of the Northeast's agricultural economy. Greenhouse ornamentals represent an important option for diversification to supplement traditional crop production<sup>32</sup>. Ornamentals are grown for their esthetic value, and thus growers can't allow pests to cause significant damage. For this reason chemical pesticides are commonly used repeatedly to control WFT and other persistent pests. Yet greenhouse growers' heavy reliance on chemical insecticides is neither sustainable nor desirable from an economic, human-health, or environmental perspective. Given the evidence of insecticide resistance in WFT, a chemical approach will ultimately fail. Growers are eager to use biologicals rather than chemical pesticides as part of IPM, but they must be assured that these alternatives work and are cost-effective. We will test a habitat plant system combining a granular formulation of entomopathogenic fungi and predatory mites on trap plants that naturally attract WFT. It offers a unique approach to sustain biological control agents when pest populations are low or absent, using fungi for the below-ground stages of the pest, and predators for the foliar phases.

**Background. Stakeholder needs.** This project directly addresses several specific needs identified by growers and other stakeholders in the Northeastern Region and nationwide. In a report of the Specialty Crop Committee of the National Agricultural Research Extension, Education and Economics Advisory Board in 2006, the authors listed ten key recommendations, two of which are directly addressed with this proposal: 1) "Alternatives to chemical crop protection, research to lower toxicity of chemicals, and work to improve biologic controls should be vigorously pursued." 2) "Emerging and rapidly growing segments of the industry such as organics, the nursery and green industry, and controlled-environment production deserve more

focus.”<sup>33</sup>. In 2006 the Northeast Research, Extension, and Academic Program Committee for IPM (NEREAP) identified areas of emphasis for IPM in the Northeastern Region, one of which was “Improvement and expansion of biocontrol in high value crops including greenhouse.”<sup>34</sup>. This project specifically relates to subjects listed in The General IPM Priorities for the Northeast, which were identified from a November 2006 poll of the Northeastern IPM Center's advisory council, state network project leaders, IPM working group leaders, and state IPM Coordinators and other NEREAP members. Specifically it relates to the general area of Biocontrol, the highest priority being “Research on biological control of diseases, arthropods, and weeds; extension of this research into production systems of horticultural crops”. It also addresses a priority for the Greenhouse Crops area: “Integration of biological controls into greenhouse ornamental pest management.”<sup>35</sup>. GO-IPM, the greenhouse and ornamentals commodity work group of the NE IPM Center, conducted a grower survey in 2002, from which several needs and priorities were identified, including: “Develop alternative, less toxic pest management tactics, including biological and cultural methods for key weed, disease, and insect pests,” and “Develop effective and easy to use application technologies for biological and chemical control.”<sup>36</sup>. Scientists at the UVM Entomology Research Laboratory maintain close links with growers through their annual greenhouse IPM workshops in Maine, New Hampshire and Vermont. Grower participants are asked to complete an evaluation at the end of the workshop which provides us with valuable insights into subjects that particularly interest them. These educational events are coordinated by the Greenhouse IPM Advisory Group of Northern New England, which is made up of researchers, growers and extension specialists. This group provides input on areas of research that are most needed by the ornamentals industry. In addition, several research projects are being conducted by Entomology Research Laboratory scientists in commercial greenhouses. This provides us with a direct link to growers and their needs in terms of IPM implementation.

**Review of Relevant Work.** Several biological control agents exist for use against the above and below ground stages of WFT. Nematodes and predatory mites are available commercially. Various entomopathogenic fungal species have been studied for WFT management, and preparations of *Beauveria bassiana*, *Metarhizium anisopliae* and *Lecanicillium lecanii* have significantly reduced WFT in greenhouse vegetable and floral crops under research conditions<sup>12,13,14,15</sup>. Such products have many desirable traits—they leave no toxic residues, are generally harmless to beneficials and pose minimal risk to humans and the environment<sup>16,17</sup>. Despite these positive aspects, in commercial greenhouses, fungi have provided inconsistent results for WFT control. Todd et al.<sup>18</sup> reported a reduction by 40% in WFT populations by using a clay-based wettable powder formulation of *B. bassiana* applied 3-4 times over 1 mo. In contrast, Jacobson et al.<sup>19</sup> reported that glasshouse populations of WFT were reduced by 87% with three consecutive high volume sprays or low volume mist applications of Naturalis-L or BotaniGard WP, both *B. bassiana*-based products, applied at 6-d intervals. Good spray coverage is essential for reliable pest control with mycopesticides, and our research shows that spray techniques used in commercial greenhouse crops are unlikely to provide the coverage needed to achieve success with biopesticides (Kassa, unpubl. data). In addition, efficacy may be reduced because a large portion of the WFT population is in the soil, where it is protected from direct contact with the fungal inoculum. Developing a fungal formulation targeting the soil stage could enhance efficacy by reducing the number of emerging adults, thereby breaking the WFT reproductive cycle.



**Fig. 1.** A) sporulating *B. bassiana* grains in potting mix, B) sporulating millet-based *B. bassiana* granule.

Though entomopathogenic fungi are commonly formulated as wettable powders for greenhouse IPM, they can also be formulated as granules, which have advantages over conventional sprays for control of soil insects<sup>20</sup>. Nutrients can be incorporated into granules to support fungal growth and sporulation, or baits to attract target pests<sup>21,22</sup>. Granules do not need to be incorporated throughout a potting mix for WFT control, but only in the top layer as the insects generally pupate in the upper 2 cm of soil<sup>2</sup>. In a previous project, we developed millet-based granular formulations of entomopathogenic fungi (Fig. 1), which when incorporated into the growing medium, reduced WFT emergence by >92% (Kassa, unpubl. data). In addition, *B. bassiana* and *M. anisopliae* inoculum levels increased over time, providing long term WFT control and eliminating the need for multiple applications over the season.

Good control of WFT pupae in potting compost and horticultural growing media has been reported by other researchers as well<sup>23,24,25</sup>. These results clearly demonstrate the potential of granular fungal formulations for WFT control in potting media as a component of IPM.

Fungi are not the “silver bullet”, and must be used as part of a total IPM program for WFT. Early detection is critical so action can be taken before WFT reach damaging levels<sup>13,26</sup>. When spring bedding plants are started in late Jan/Feb, 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. However, we tested marigolds as trap or indicator plants, and found that WFT were detected several weeks earlier on flowering Lemon Gem marigolds than on sticky cards (Fig. 2). Beneficials are best used as preventatives, and introduced during the early stages of an infestation when WFT adults are present in low numbers. With support from the NE IPM Grants Program, we used Lemon Gems as ‘banker plants’, which, when inoculated with predatory mites, *Neoseiulus californicus*, served as a reservoir for on-going production of natural enemies. Banker plants are used in European and Canadian vegetable greenhouses and can be a cost effective means of providing a continuous supply of beneficials<sup>27</sup>.



**Fig. 2.** Marigold indicator plant in commercial greenhouse.

This project is an innovative way to integrate multiple complementary biological control agents within a “habitat plant system” that could be effective and cost-efficient. Several terms have been used interchangeably to describe various uses of plants as IPM tools, and are defined in the context of this project in the sidebar on the following page. A CRIS search using the key words: ‘thrips, entomopathogens; indicator plants; greenhouse IPM, predatory mites; marigolds; banker plants’ netted 80 records. Only two specifically involve banker plants and natural enemies in greenhouse ornamentals. None is studying banker/habitat plants with predatory mites on marigolds in combination with entomopathogenic fungi for WFT control. One study tested castor bean as a banker plant with predatory mites; they were not particularly effective (UMASS). We found that marigolds, with a continuous supply of pollen, are more effective than beans, which favor a buildup of WFT but not predatory mites. A Cornell Univ. study used marigolds as a banker plant to support *Orius insidiosus*, a WFT predator. We tested this

previously and found *Orius* populations did not build up sufficiently to provide adequate control. Cornell Univ. scientists are also testing Botaniguard®, a *B. bassiana* foliar spray, with *Orius* and *N. cucumeris*, against WFT. They are not, however, using fungi together with marigolds as a habitat plant nor are they using a granular fungal formulation. A study is also underway to assess the compatibility of predatory mites and spinosad (Conserve), a pesticide commonly used against WFT (UMASS). They found these two WFT management tools were compatible. This indicates that Conserve could potentially be used in combination with our habitat plant system, should WFT populations build up to damaging levels. It should be noted however, that evidence of resistance to Conserve among some WFT populations has been reported (C. Frank, pers. comm.). This demonstrates the urgent need to design alternative IPM approaches that do not rely on insecticides.

Habitat plants are specifically selected to attract pests out of the crop, serving as a reservoir for low-level pest populations and their natural enemies. This system provides a continuous supply of healthy natural enemies on-site saving growers time and money<sup>40,41</sup>. As there are few producers of natural enemies in the Northeast, growers sometimes have difficulties obtaining beneficials when they need them; these also must be shipped from producer to distributor (where they may be re-packaged) to grower, so their quality can be compromised. Another advantage of habitat plants is that the natural enemy population on plants may become acclimatized to the local environment over time, and will perform more efficiently than those reared elsewhere. Lastly, if growers can establish a breeding population of natural enemies in their greenhouses, they will save money over the season. The major disadvantage of habitat plants is that, if not managed carefully, the resident pest population can increase there and spread to plants grown for sale<sup>42</sup>. We observed a similar problem when testing marigolds as indicator plants. While WFT were detected early on the marigolds, without suitable biological controls in the system, the pest built up on the plant which had to be removed from the growing area.

Whereas habitat plants have been shown to be effective in greenhouse and field-grown vegetables, their efficacy has not been widely assessed in greenhouse ornamentals. Flowering castor bean plants were used to colonize the predatory mite *Amblyseius degenerans* for thrips control in a greenhouse crop of sweet peppers. After 4 wks, the mites had dispersed up to 53 plants away from the banker plant, though migration across rows was limited<sup>43</sup>. The economics of using banker plants in Canada was investigated for management of several different pest species in a variety of greenhouse-grown vegetable crops; biocontrol costs were reduced by 75% when banker plants, rather than regular inundative releases, were used<sup>40</sup>. We are currently evaluating eggplant as habitat plants in poinsettias in association with parasitic wasps and to date, pesticide sprays have been cut in half in greenhouses where this system is being tested. This novel technology shows great promise, but further study is needed to expand their use in greenhouse ornamentals.

### **Plant Type Definitions**

**Indicator plant:** Attracts pests and natural enemies serving as an early detection tool.

**Trap plant:** Highly attractive to pests, drawing them away from the crop where a spot spray or biocontrols can be applied.

**Banker plant:** Supplies prey (non-pest species) to support a continual supply of beneficials that disperse into the crop.

**Habitat plant:** Blend of above plant types: attracts pests out of the crop and serves as an early pest detector; functions as a reservoir for biocontrol organisms, providing food (pollen/prey), oviposition sites and shelter to beneficials and a suitable environment for microbial colonization.

**Justification.** Growers will be the immediate beneficiaries of the results of this research through the development and testing of a novel IPM method that maximizes the potential of entomopathogenic fungi and predatory mites within a sustainable habitat plant system. This system could reduce the need for repeated fungal applications or predatory mite releases, which would save growers time and money. It would also lessen or eliminate growers' reliance on chemical control for WFT. The compounds commonly used against WFT pose a significant risk to applicators, consumers and the environment, and repeated pesticide applications can adversely impact plant physiology and appearance<sup>37,38,39</sup>. Therefore, not only growers but the public at large will benefit from a reduction in the use of chemical pesticides on ornamentals.

Through this project we will combine multiple IPM tactics together that will serve as both a pest detection tool and a management strategy. This is a cost effective and efficient approach to WFT control. Too often research focuses on one aspect of control, yet growers must deal with a variety of issues for successful plant production. This project directly applies to several priorities of the Regional IPM Competitive Grants Program for the Northeastern Region. Specifically, as stated in the Background section, it addresses stakeholder priorities as identified by multiple agencies and organizations. Results we generate through this project will be applicable to greenhouse ornamentals throughout the Northeastern Region, but will also be suitable for greenhouse production throughout the US. As stated above, the greenhouse ornamentals industry is a multi-billion dollar crop, and thrips is one of the most persistent, widespread pests that contribute significantly to reduced revenues. Pesticide resistance to WFT has been reported and growers need biological control solutions now to replace their traditional chemical control approach. Because we are testing biological control agents that are currently available commercially, implementation of this habitat plant system should be relatively rapid. Considering the simplicity of the system, it is likely that growers would readily adopt it if it is found to be as or more effective than chemical pesticides. Cost and availability are often cited as factors limiting growers' use of natural enemies. Habitat plants provide a constant supply of predators into a crop, reducing the need for multiple releases, and ensuring that a resident population is present when a pest outbreak occurs. Few inputs are needed to achieve long-term suppression once the natural enemy is established. The cost of shipping natural enemies is often higher than the cost of the beneficials themselves; when multiple releases are required, shipping costs become a major expense, which can be minimized by using habitat plants.

## **ii. Objectives and Anticipated Impacts**

**Obj. 1. Assess the impact of soil applications of granular formulations of insect-killing fungi on the population dynamics of western flower thrips.**

**Obj. 2. Evaluate the combined effect of a granular fungal formulation and predatory mites within a habitat plant system to prevent the buildup of western flower thrips populations.**

We propose to combine predatory mites, granular entomopathogenic fungi and indicator plants into one effective package for WFT IPM in greenhouses, which for this project is termed a "habitat plant" (HP). Adult WFT will be attracted to the marigolds, where a small population will become established. A portion of the resultant immature WFT on the plant will serve as prey for the predatory mite, *N. cucumeris*, sustaining the mite population, and encouraging its dispersal throughout the greenhouse over time. A portion of the WFT that escape predation will

drop to the soil to complete development, where they will be targeted with fungi. The granular formulation will enable the fungus to colonize the potting mix, and eliminate the need for repeat applications. This concept represents a sustainable, low-cost, environmentally sound approach to combating the most serious pest problem facing greenhouse growers today. Table 1 below describes the anticipated impacts that will result from this project.

**Table 1. Projected impacts of this research project**

Type of impact	Potential impact
Safeguarding human health and the environment	<ul style="list-style-type: none"> <li>- The health of pesticide applicators and other production staff who are commonly exposed to pesticide residues in ornamental crops will be safeguarded by devising an effective biological control approach.</li> <li>- Chemical sprays are applied repeatedly on greenhouse ornamentals to control WFT. These applications will be reduced with the use of habitat plants thereby reducing the exposure to pesticide residues by the public who enter retail greenhouses and come in contact with ornamentals.</li> </ul>
Economic benefits	<ul style="list-style-type: none"> <li>- New specific chemical pesticides are becoming increasingly expensive, whereas the cost of biological control agents is declining as the demand increases.</li> <li>- Damage and economic loss caused by WFT will be reduced significantly by using cost effective biological control strategies.</li> </ul>
IPM implementation	<ul style="list-style-type: none"> <li>- Over 60% of growers attending our greenhouse workshops are using biological control, and all express interest in expanding their use of IPM. Therefore if we demonstrate the effectiveness of habitat plants for WFT IPM, a high percentage of growers in our region will certainly use them.</li> <li>- There are thousands of greenhouse growers in the Northeastern Region, and without a doubt, all of them struggle with WFT. All of these growers would adopt this IPM strategy if they knew it worked and it cost less than chemical pesticides.</li> <li>- By demonstrating the potential of granular fungal formulations for WFT IPM, private companies that currently produce foliar sprays will be encouraged to expand their product line to include granules.</li> </ul>

Through this project we will conduct research on a novel IPM approach for management of WFT. It is unrealistic to anticipate that it will be implemented by growers within the project period. First we need to demonstrate its effectiveness. Thus we cannot expect a measurable decrease in pesticide use to occur immediately. However, once the Habitat Plant system is refined and perfected, it is likely to be broadly adopted. We have observed growers' eagerness to use similar banker plant systems for aphid IPM and expect a similar response, especially if WFT develops broader resistance to currently available chemical pesticides. A logic model in Section iv below has been developed to further illustrate the short and long term impacts of this project.

### iii. Approach and Procedures

**Obj. 1. Assess the impact of soil applications of granular formulations of insect-killing fungi on the population dynamics of western flower thrips.**

**Source and production of fungi.** *Beauveria bassiana*, strain GHA, will be used for the study. GHA is contained in a commercially-available fungal-based foliar spray product, BotaniGard® (Emerald BioAgriculture Corp., MT) used by growers to manage WFT. A granular formulation of this isolate is not available commercially so we will produce it for these tests. The strain is preserved at the UVM Entomology Research Laboratory (ERL) and the USDA, ARS collection. Conidia will be produced on quarter strength sabouraud dextrose agar yeast medium (SDAY) at 25°C and maintained at 4°C. Conidia from 12-d old cultures will be used for inoculation of the liquid culture for mass production. A millet-based mycelium granule formulation will be produced using methods developed at the ERL (Kassa *et al.*, unpublished).

**Arena study (Year 1).** Through this experiment we will evaluate the efficacy of the millet-based granular formulation of GHA alone, which will establish the basis for trials outlined in Obj. 2. Three application rates of the GHA granular formulation will be compared with the standard biopesticide (BotaniGard®) and untreated controls (Table 2) in the laboratory under controlled simulated greenhouse conditions.

**Table 2. Fungal treatments**

Trt.	Treatment
1	Millet-based granular formulation of <i>B. bassiana</i> , GHA strain, 3 g/pot (one time application)
2	Millet-based granular formulation of <i>B. bassiana</i> , GHA strain, 5 g/pot (one time application)
3	Millet-based granular formulation of <i>B. bassiana</i> , GHA strain, 8 g/pot (one time application)
4	Standard foliar spray-application of BotaniGard (3-4 applications/season, as recommended)
5	Untreated control, no fungal treatment

Lemon Gem marigolds will be used as the target plant because it is highly attractive to WFT and can serve as an indicator plant (Skinner & Frank, unpublished). Pollen produced in the flowers also will support predatory mites in the absence of thrips. For each treatment, 12 cylindrical cages (50 cm diam., 50 cm tall) covered with thrips-proof mesh will be set up, each containing a potted marigold in flower. Prior to placing in the cage, each plant will be sampled for WFT by lightly tapping it over a white sheet of paper (10 taps/plant) to dislodge arthropods. The number of adult and immature WFT will be counted, and then returned to the plant. We found this sampling method provided a reliable assessment of WFT populations on marigolds without disrupting the population dynamics significantly. After sampling, 25 adult female WFT from the ERL stock culture will be released onto each plant and left undisturbed for 2 wks. Plants will then be sampled again as described above to determine the baseline WFT population per plant, after which treatments will be applied.

Fungal-based granules will be sprinkled around the base of the growing plant at the rate of 3, 5 and 8 g/pot, which after 9-12 d will provide a spore concentration of  $\sim 3.6 \times 10^8$ ,  $6 \times 10^8$ , and  $.6 \times 10^9$  spores/g of potting medium in the top 2 cm (1g granule  $\sim 1.2 \times 10^8$  conidia). BotaniGard® will

be sprayed as recommended by the producer. The controls will be untreated. The growing mix will be kept moist throughout the experiment by bottom-watering. Plants will be sampled every 2 wk for 10 wk to assess WFT populations. Samples of dead WFT will be collected at random on the foliage, surface sterilized, placed in a moist chamber and incubated at 25°C for 10 d. The presence/absence of entomopathogenic fungi will be recorded according to identification criteria of Humber<sup>28</sup>. Leaf and flower damage will be rated at each sampling date using a 0-5 scale (0=no damage, 1=WFT feeding damage on <10% of the plant, 2=feeding on 10-25% of the plant, 3=damage on 26-50% of the plant, 4=damage on 51-75% of the plant, 5=feeding on 75-100% of the plant). Ambient temperature will be ~25°C, similar to that in a greenhouse. Data on environmental conditions within the cages (temp. and RH) will be recorded throughout the study. The experiment will be arranged in a randomized complete block design with 12 replications and the entire study will be repeated twice per season.

**Statistical analysis.** Separate analyses of variance will be made for each sample date on the square root-transformed numbers of WFT per plant. Data will be analyzed using the general linear model<sup>29</sup>. The percentage of population reduction (relative to the untreated controls) for each treatment will be used to identify the most effective treatments. The plant damage data will be analyzed using the general linear model following a square-root transformation. In the event of significant differences, comparisons among means in all parameters will be made using Student-Newman-Keuls (SNK) test<sup>29</sup>.

**Obj. 2. Evaluate the combined effect of a granular fungal formulation and predatory mites within a habitat plant to prevent the buildup of western flower thrips populations.**

**Caged study (Year 2).** Based on results from Obj. 1, we will select the application rate for the granular fungal formulation with the highest efficacy against WFT to test in association with the predatory mite, *N. cucumeris*. Eighteen cages constructed with thrips-proof mesh will be set up, each containing four potted marigolds in flower. The same basic research methods will be followed as described for Obj. 1, testing six treatments (Table 3).

**Table 3. Fungal and mite treatments**

Trt.	Treatments
1	Untreated control, no fungi or mites applied (sterile millet seeds alone)
2	Untreated control with predatory mites only (sterile millet seeds & mites)
3	Millet-based granular fungal formulation of <i>B. bassiana</i> GHA strain without mites
4	Millet-based granular fungal formulation of <i>B. bassiana</i> GHA strain with mites
5	Standard foliar spray-application of BotaniGard without mites
6	Standard foliar spray-application of BotaniGard with mites

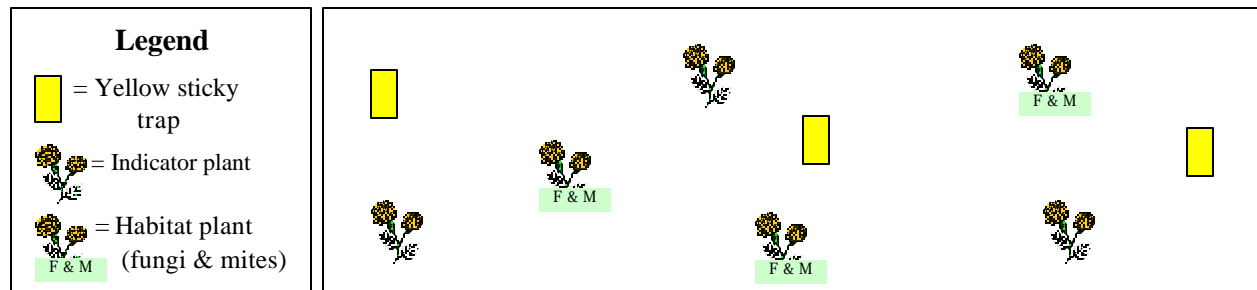
Predatory mites will be introduced on to the plant by attaching one commercially-available sachet per plant at the same time WFT are released. These sachets contain both predatory mites and grain mites, which serve as prey for the predators. The sachets act as a slow release mechanism, providing a steady supply of predators over 6 wk. We anticipate the predatory mite population will be naturally sustained after that on flower pollen and WFT. The plants will be sampled every 2 wk, as for Obj. 1. Prior to conducting the whole-plant sampling (plant tapping), three marigold flowers will be destructively sampled from each plant. This will ensure that we

obtain an accurate estimate of the mites, which may not be dislodged when the plant is tapped because they hide deep in the flowers. Fungal treatments will be applied 2 wks after the WFT release. Each treatment will be replicated three times (3 cages/treatment, 4 plants/cage) and the entire study will be repeated twice per season, making a total replication of six per treatment.

To determine the potential predatory mite population that will be released, when sachets are placed on the caged plants, four separate sachets will be destructively sampled and the number of predatory and grain mites will be counted. In addition, two cages, each containing four marigolds infested with WFT but not treated with fungi, will be set up. Predatory mite sachets will be placed on each plant and destructively sampled after 5 and 10 wk. Plants and flowers will also be sampled for WFT and mites as above. This will provide valuable information on how long a sachet will sustain predatory mites for release, and what proportion of the mites in the sachet disperse onto the plant. Data on the WFT and mite populations will be analyzed statistically according to methods described in Obj. 1.

**Greenhouse trial (Year 3).** We expect the granular formulation will outperform or at least provide results comparable with the spray formulation in terms of reducing WFT population increase over time. Therefore, in the greenhouse trial we will assess the combined effect of the granular fungal formulation, and *N. cucumeris* within the marigold habitat plants on WFT population levels in commercial greenhouses. Two private growers who produce spring bedding plants have been enlisted to cooperate on the project. Two plant treatments will be tested: marigolds with a granular fungal formulation and predatory mites (habitat plants) and marigolds alone (indicator plants). Yellow sticky traps will be considered a treatment as this is a standard method used by growers to detect WFT in greenhouses. Data from the traps will allow us to compare trap catches with plants in terms of their ability to detect WFT.

Marigolds will be produced from seed in an ERL greenhouse to ensure clean plant material. When production of spring bedding plants begins (late Feb.-Mar.), we will place six marigolds into the greenhouses (3/treatment), spaced at least 2 m apart (Fig. 3). At the time when plants are placed in the greenhouses, predator mite sachets will be hung on the habitat plant treatment. In addition, the required amount of the granular fungal formulation will be sprinkled around the base of each of these plants. Because this trial will take place in commercial greenhouses, we will rely on a natural buildup of WFT in the greenhouses. Based on our previous trials and the pervasiveness of WFT, we have no doubt an infestation will occur.



**Fig. 3.** Diagram of trial setup in a commercial greenhouse.

WFT and *N. cucumeris* numbers will be counted weekly for 10 wk beginning on the date they are placed in the greenhouses using the sampling method described above. Leaf damage rating will also be done as described in Obj. 1. Every 2 wk, three blossoms will be destructively sampled to further assess residual mite and WFT populations. The number of WFT will also be counted on the yellow sticky traps. This entire experiment will be replicated twice during the growing season each for the 10-wk period, making the total replication six per greenhouse and 12 in total (2 greenhouses x 6).

We recognize that all treatment combinations are not represented in this greenhouse trial, i.e., indicator plants with only mites or only fungal granules. However, this trial is meant to test the best management option in the “real world”. We will interact closely with the grower cooperators so that we are fully informed about pesticide applications the growers feel obligated to make. If necessary, our marigolds will be removed during spraying so mite populations are not negatively impacted by the spray.

**Statistical analysis.** Analysis for the caged trial will be completed as described for Obj. 1, with separate analyses of variance for each sampling date on the square root-transformed numbers of WFT and predatory mites recorded per plant. Leaf damage data will be analyzed as described above.

For the greenhouse study, the different greenhouses will be considered as a main plot (site) and the treatments will be considered as subplots. A square-root transformation will be made on the thrips (on plants and yellow sticky traps) and mite data and subjected to statistical analysis using a two-factor randomized complete block design<sup>29</sup>. The leaf damage data will be analyzed using a general linear model following an arcsine transformation. In the event of significant differences, comparisons of means for all parameters will be made using the Student-Newman-Keuls (SNK) test<sup>29</sup>.

**Timetable.** The projected effective dates of project: Oct. 1, 2008 – Sept. 30, 2011

Obj.	Task/Activity	Completion date
1	Produce marigolds for habitat plant system	Oct. 2008
Yr. 1	Mass produce granular fungal formulations for testing	Oct. 2008
	Conduct 2 replications of the fungal concentration trial	Dec. 2008-Jun. 2009
	Compile data and analyze results, and prepare report	Sept. 2009
	Prepare manuscript for refereed journal	Sept. 2009
2	Produce marigolds for habitat plant system	Oct. 2009
Yr. 2	Mass produce granular fungal formulations for testing	Oct. 2009
	Conduct 2 replications of habitat plant system	Dec. 2009-Jun. 2010
	Compile data and analyze results and prepare report	Aug. 2010
Yr. 3	Produce marigolds for habitat plant system	Oct. 2010
	Mass produce granular fungal formulations for testing	Oct. 2010
	Conduct greenhouse trial of habitat plant system	Jun. 2011
	Compile data and analyze results and prepare manuscript and final report.	Aug. 2011

#### **iv. Evaluation Plans**

This is a research project that will investigate the effectiveness of a novel IPM strategy utilizing multiple IPM tools combined into one habitat plant system, i.e., indicator plants for early pest detection, and predatory mites and entomopathogenic fungi for biological control. We anticipate that this habitat plant system will provide sustained management of WFT in spring bedding plants. The research design outlined in the Approach and Procedures section will enable us to evaluate the potential of this system for WFT IPM, both within a controlled laboratory environment and in a commercial greenhouse setting. It will provide data on the most efficacious rate of a granular fungal formulation to use to reduce WFT populations. It will demonstrate the combined effect of fungi and predatory mites on WFT population dynamics and compare the effect of granular vs foliar applications of a commercially available fungus. These results will lead to more widespread testing of the system in commercial greenhouses before implementation by growers would be encouraged.

It is unrealistic to expect that this system would be implemented before we have fully investigated its potential. We do anticipate presenting our research results to growers through the annual Northern New England Greenhouse IPM workshops. This will ensure that growers are prepared to implement the system when it is found to be effective. It will also ensure that the system is compatible with existing production practices. The interactive format of these workshops will allow us to obtain feedback on the strengths and barriers that growers would anticipate relevant to implementation of the proposed habitat plant system. No costs will be incurred from this project to disseminate the results to growers.

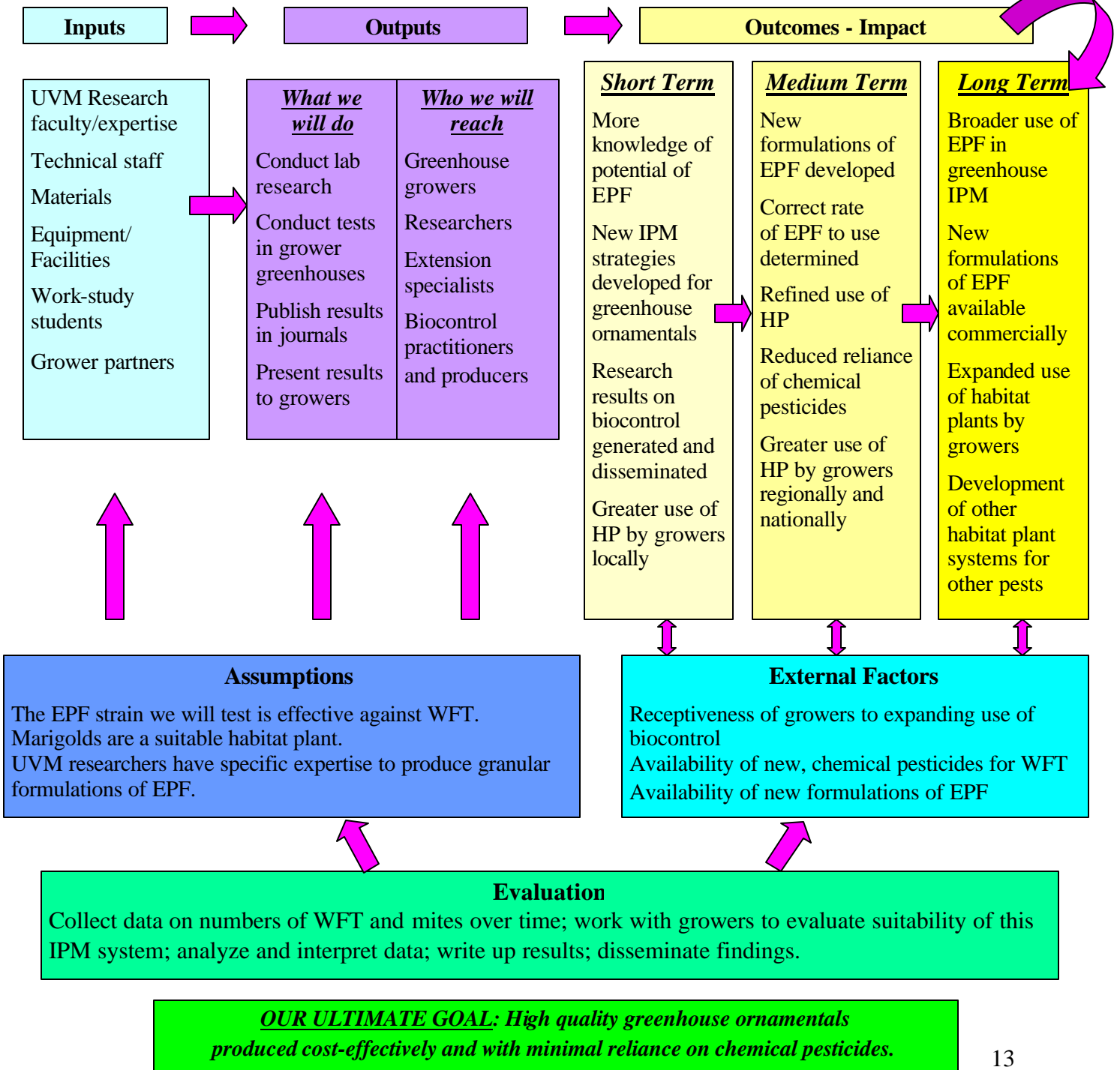
The habitat plant system would be seamlessly incorporated into existing greenhouse IPM practices. It is completely compatible with existing production systems and would only enhance their on-going pest detection and management programs. It might be necessary for growers to remove the habitat plants when they make a pesticide application to ensure that the natural enemies are not affected. However, depending on the type of spray equipment used and the compound applied, it may be sufficient for growers to merely spray around the habitat plants.

For growers to use the habitat plant system we are testing, companies that are currently producing entomopathogenic fungi would have to offer granular formulations. If our results consistently demonstrate the promise of granular fungal formulations, we will interact with these companies and encourage them to expand their product line to include them. Below is a Logic Model which summarizes the inputs, outputs and impacts of this project.

**Logic Model**

**Situation:** Chemical pesticides are used extensively to control western flower thrips (WFT) in greenhouse-grown bedding plants. Resistance to many pesticides currently used against WFT has been reported. Soil phases of WFT escape the effects of most chemical and biological control treatments. Entomopathogenic fungi (EPF) show potential against WFT, but are underutilized.

**Priorities:** Develop novel IPM strategies for WFT using Habitat Plant Systems (HP) that expands effective use of multiple biological control agents.



## **v. Key Personnel**

Margaret Skinner, Ph.D., Entomologist and Extension Specialist, (Principal Investigator): She will coordinate research activities, manage administrative responsibilities, assist with treatment evaluation, prepare the required reports and participate in outreach activities to disseminate results to growers.

Adana Kassa, Ph.D., Insect Pathologist, (Co-Principal Investigator): He will take a leadership role in setting up and conducting the planned research, including treatment evaluation, analysis of the data and preparing manuscripts.

Svetlana Gouli, Ph.D., Microbiologist: She will take full responsibility for mass producing the fungi for testing, including preparation of the granular formulations and spore powders.

### **Why Does This Project Deserve Support?**

- It specifically addresses several priorities identified by the NE IPM Center, other regional and national IPM entities and most importantly growers.
- It is a grower participatory research project that enlists cooperation from the end user, which will hasten implementation of the results.
- It tests an innovative IPM strategy combining two biocontrol agents into a package that could provide synergistic sustained ecological management.
- It addresses the needs of an underserved target audience, i.e., growers of small acreages of specialty crops.
- The scientific team doing the research has a proven record of conducting sound practical research that addresses grower needs; a team with a demonstrated ability to transfer technology resulting in greater IPM implementation.
- The project addresses the #1 pest in the production of greenhouse ornamentals, against which chemical pesticides are heavily used nationally.
- It could revolutionize IPM of western flower thrips, providing non-chemical options for a pest that has developed insecticide resistance.
- It addresses needs of an agricultural sector that annually generates billions of revenue dollars for growers nationally.
- It focuses on crops that offer viable options for preserving family farms in New England through crop diversification.
- Results could contribute significantly to reducing chemical insecticide use in a crop known as one of the US's heaviest pesticide users on a per acre basis.

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## Relevance Statement

- a. Project Directors:** Margaret Skinner, Adana Kassa  
**Institution:** Entomology Research Laboratory, The University of Vermont  
**Cooperators:** Ron Paquette, Paquette Full of Posies; Chris Conant, Claussen's Greenhouses
- b. Title:** Fungi, Predatory Mites and Habitat Plants for Thrips IPM in Greenhouse Ornamentals
- c. Project Type:** Research
- d. Project Summary:**

This is a Research project testing a novel approach for IPM of western flower thrips (WFT) in spring bedding plants that combines predatory mites, granular entomopathogenic fungi and indicator plants into one effective "habitat plant system". Adult WFT will be attracted out of the crop to flowering marigolds, where they will become established. A portion of the resultant immature WFT on the plant will serve as prey for the predatory mite, *Neoseiulus cucumeris*, sustaining the mite population, and encouraging its dispersal throughout the greenhouse over time. A portion of the WFT that escape predation will drop to the soil to pupate, where they will be targeted with fungi. The granular formulation will enable the fungus to colonize the potting mix, eliminating the need for repeat applications. This concept represents a sustainable, low-cost, ecological approach to combating the most serious pest facing greenhouse growers today. This project will contribute to safeguarding human health and the environment by reducing growers' use of chemical insecticides, thereby reducing exposure of applicators, growers, agricultural workers and the public to toxic compounds. This study tests an innovative IPM strategy that could offer economic benefits to growers by reducing plant damage from WFT, increasing plant quality, and minimizing production costs by providing a sustained source of biocontrol agents within the crop. This could lead to wider adoption of IPM by growers, thousands of whom in the Northeast struggle annually with WFT control. Growers would surely adopt this IPM strategy if we demonstrate it works and costs less than chemical control.

**e. Problem, Background & Justification.** Western flower thrips (WFT) remain one of the most serious pests of protected agriculture in the US and world-wide. They are identified by growers as a primary pest throughout New England, where in some crops, pesticides are applied every 3-7 d. WFT feeds on a wide range of greenhouse ornamentals, causing cosmetic damage that reduces crop value and transmitting virus diseases, which can destroy an entire crop. Its cryptic behavior, rapid reproductive rate and potential to develop resistance make WFT particularly difficult to control. Predatory mites are used to combat WFT in the foliar parts of the plant, but many pupate in the soil, escaping most management tactics. Formulations of entomopathogenic fungi are available for foliar application against WFT but are not widely used and reports of their efficacy have been inconsistent. Granular fungal formulations show promise in laboratory trials but are not currently available commercially.

### f. Project Objectives & Anticipated Outcomes

- Obj. 1.** Assess the impact of soil applications of granular formulations of insect-killing fungi on the population dynamics of western flower thrips.
- Obj. 2.** Evaluate the combined effect of a granular fungal formulation and predatory mites within a habitat plant system to prevent the buildup of western flower thrips populations.

## How does this project stack up to the RIPM Relevancy Rating?

1. It is not a multistate partnership because that would not contribute to achieving the project objectives at this time. First we need to demonstrate the potential of habitat plants under controlled laboratory conditions, and in limited greenhouse trials. That is best done at our research facility where we can cost-effectively evaluate the results. The outcome of these trials will determine if it is appropriate to expand the work to other states.
2. This project directly addresses priorities identified by these committees and IPM entities:
  - A. Specialty Crop Committee: Nat. Agric. Res. Ext., Ed. & Econ. Advisory Board, 2006.
  - B. Northeast Res., Ext. & Academic Program Committee for IPM (NEREAP) identified areas of emphasis for IPM in the Northeastern Region
  - C. General IPM Priorities for the Northeast, identified from a Nov. 2006 poll of the Northeastern IPM Center's Advisory Council, state network project leaders, IPM working group leaders, and state IPM Coordinators and other NEREAP members.
  - D. 2002 grower survey by GO-IPM, greenhouse and ornamentals commodity work group of the NE IPM Center.
3. This targets an underserved audience, specifically New England growers with small acreages of specialty crops (greenhouse ornamentals). They manage relatively small operations and have unique needs not addressed by much of the national research initiatives that focus on huge greenhouse operations on the West Coast. Results from this project will specifically apply to greenhouse growers in the Northeast region, though they will be applicable nationwide.
4. WFT is not a new pest in greenhouse ornamentals, but it remains one of the most persistent and challenging problem. The fact that resistance to the most commonly used pesticide, Conserve, has been reported will exacerbate the problem.
5. WFT is a pest of great National and International significance that transmits a serious disease in ornamentals. It is safe to say that no greenhouse in the US can escape this pest.
6. The annual wholesale value of greenhouse ornamentals in New England in 2004-05 is >\$460 million, a 17.5% increase since 2000. The total annual wholesale value nationally is \$14 billion.
7. See #4 and 5 above. If growers lose Conserve for chemical control, IPM is even more critical.
8. Results could be implemented within 1-2 years if granular fungal products are available.
9. This integrates 3 disciplines, pest detection, biocontrol with predators and microbial control
10. This is a full integrated use of sustainable production practices, including a plant for early detection that attracts the pest from the crop, two biological control agents to reduce populations and a system that sustains these agents over time, eliminating the need for reapplications.
11. As stated in the summary above, the approach we will test clearly addresses the mission of the NE IPM Center in terms of reducing human exposure to pesticides, protecting the environment from pesticide contamination, and devising cost-effective sustainable IPM approaches that decrease the need for multiple releases of biologicals and lessen crop damage.
12. We have shown that habitat plant systems in poinsettias can reduce pesticide use by over 50%. Similar results in bedding plants are possible using the habitat plant system we propose.

**Logic Model**

**Situation:** Chemical pesticides are used extensively to control western flower thrips (WFT) in greenhouse-grown bedding plants. Resistance to many pesticides currently used against WFT has been reported. Soil phases of WFT escape the effects of most chemical and biological control treatments. Entomopathogenic fungi (EPF) show potential against WFT, but are underutilized.

**Priorities:** Develop novel IPM strategies for WFT using Habitat Plant Systems (HP) that expands effective use of multiple biological control agents.

**Inputs** → **Outputs** → **Outcomes - Impact**

UVM Research faculty/expertise  
 Technical staff  
 Materials  
 Equipment/Facilities  
 Work-study students  
 Grower partners

<u>What we will do</u>	<u>Who we will reach</u>
Conduct lab research	Greenhouse growers
Conduct tests in grower greenhouses	Researchers
Publish results in journals	Extension specialists
Present results to growers	Biocontrol practitioners and producers

<u>Short Term</u>	<u>Medium Term</u>	<u>Long Term</u>
More knowledge of potential of EPF	New formulations of EPF developed	Broader use of EPF in greenhouse IPM
New IPM strategies developed for greenhouse ornamentals	Correct rate of EPF to use determined	New formulations of EPF available commercially
Research results on biocontrol generated and disseminated	Refined use of HP	Expanded use of habitat plants by growers
Greater use of HP by growers locally	Reduced reliance of chemical pesticides	Development of other habitat plant systems for other pests
	Greater use of HP by growers regionally and nationally	

**Assumptions**  
 The EPF strain we will test is effective against WFT.  
 Marigolds are a suitable habitat plant.  
 UVM researchers have specific expertise to produce granular formulations of EPF.

**External Factors**  
 Receptiveness of growers to expanding use of biocontrol  
 Availability of new, chemical pesticides for WFT  
 Availability of new formulations of EPF

**Evaluation**  
 Collect data on numbers of WFT and mites over time; work with growers to evaluate suitability of this IPM system; analyze and interpret data; write up results; disseminate findings.

**OUR ULTIMATE GOAL:** High quality greenhouse ornamentals produced cost-effectively and with minimal reliance on chemical pesticides.