Development of new insect suppression solutions for greenhouse production

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Abstract

Over the past few years, the biological control of greenhouse pests has greatly evolved to offer solutions that are more environmentally friendly and better for non-targeted organisms (pesticide-free), but also safer for workers directly exposed to those control products. A clear majority of the solutions developed to this day are based on the use of beneficial insects (predators or parasitoid), entomopathogenic fungi and bacteria. However, using them can be complex and intensive and requires extensive monitoring. Microorganism-based bioinsecticides, such as *Bacillus* spp., have the advantage of being easy to use. Bacteria *Bacillus pumilus* and *Bacillus subtilis* are known for their antifungal action but their potential as an insecticide has yet to be studied. The main objective of this study is to evaluate the efficacy of *B. pumilus* for the control of western flower thrips and sciarid flies. The experiments were conducted in greenhouse environment and on various plant models (pepper, cyclamen, begonia, petunia and broccoli). With this study, we showed the insect suppressive effect of *B. pumilus* PTB180 for the control of sciarid flies when the bacterium is used as a growing medium amendment.

Keywords: bioinsecticide, *Bacillus* spp., *Bacillus pumilus*, growing media, biological control, pest control

INTRODUCTION

During the past decades, synthetic pesticides have been widely used to manage insects and fungi affecting ornamental and food crops. The intensive use of pesticides has led to preoccupying issues such as pesticide resistance in insect pests and microbial pathogens, health concerns for workers and consumers, toxicity for beneficial or non-targeted organisms and environmental hazards. In this context, demands have risen for better pest management solutions or complementary strategies to reduce synthetic pesticide uses. Biopesticides (living organisms and their components) are a promising alternative to synthetic chemical pesticides. They are mostly target specific, easily biodegradable and safer for human health and the environment than the conventional pesticides (Chattopadhyay et al., 2017; Miller et al., 1983). They can also be useful in situations where chemical pesticides cannot be used due to residue concerns or organic certification. Nonetheless, using biopesticides can sometimes be complex and intensive. Extensive monitoring is required because biopesticide control measures are more effective when pest infestations are still at an early stage, before populations start to increase. Moreover, extensive knowledge of the relation between the organisms involved (prey and predator or pathogen) is often needed (Fravel, 2005).

Over the past few years, great progress has been made in biological control development for greenhouse pest management (Chattopadhyay et al., 2017). Most of the solutions developed are based on the use of various beneficial insects (predators or parasitoid), entomopathogenic fungi (e.g., *Beauveria bassiana*, *Trichoderma harzianum*) and bacteria (e.g., *Bacillus thuringiensis*) (Chandler et al., 2011; Cloyd, 2008, 2015; Pérez-García et al., 2011). Although a limited quantity of products is available on the market today, a much larger quantity of naturally occurring microorganisms or microbial by-products have been identified as potential biopesticides agent (Miller et al., 1983; Chattopadhyay et al., 2017).

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More than 100 bacteria species have been reported as presenting entomopathogenic activity (Miller et al., 1983; Chattopadhyay et al., 2017), but only few have been examined closely in the past decades. Bacteria from *Bacillus* genus have interesting characteristics for potential biocontrol agents and plant growth promoting bacteria. Many are known to produce different metabolites and compounds that have fungicide and insecticidal effects (Aydi Ben Abdallah et al., 2017; Jacobsen et al., 2004; Rishad et al., 2017; Tozlu et al., 2011). Moreover, *Bacillus* are endospore forming bacteria that can resist to unfavorable environmental conditions and that can be easily formulated and stored as commercial products (Pérez-García et al., 2011; Fravel, 2005). Spore forming bacteria have also the advantage of being easy to use. They can be easily incorporated into growing media by adding concentrated formulations directly into substrates during manufacturing or by being drench on potted plant substrates.

Bacillus pumilus and *Bacillus subtilis* are already well-known for their antifungal action (Leifert et al., 1995; Pérez-García et al., 2011) but their potential as insecticide agents has yet to be studied. Few information is currently available in literature on the effect of these *Bacillus* on insects, but some strains of *B. pumilus* have already showed interesting results. Rishad et al. (2017) found that *B. pumilus* MCB-7 had a high chitinase (a cell wall-degrading enzyme) production capacity and showed suppressive effects against larvae of *Scirpophaga incertulas* in laboratory conditions. Another laboratory study in petri dish have found similar results with another *B. pumilus* strain (FDP-32) against *Bruchus dentipes* (Baudi) (Tozlu et al., 2011). The aim of the present study is to evaluate the efficacy of different *Bacillus* strains for the control of two important economic insect pests in greenhouse production: sciarid flies commonly called fungus gnats (*Bradysia* spp.) and western flower thrips (*Frankliniella occidentalis*).

Western flower thrips are generally the predominant species of thrips found in greenhouses. They are mainly phytophagous insects causing damages on aerial parts of horticultural crops such as leaves, flowers, stems and fruits (Fraval, 2006; Murphy et al., 2014). Although their life cycle mainly take place on the plant, prepupal and pupal stages often complete their development on growing medium (Murphy et al., 2014).

Fungus gnats mainly affect cuttings and young plants. Each adult female deposit 100 to 200 eggs into fissures and cracks of moistened growing media, leading to the hatching of legless larvae that lives within the top 2.5 and 5.0 cm of substrate until their development into mature adults (Cloyd, 2008). During larval stage, fungus gnats are principally microphagous, but also feed on organic matter, decaying plant matter and plant roots when there is food shortage (Kühne and Heller, 2010). Fungus gnats larval stage is responsible for most damages on controlled environment crops by feeding on roots and tunneling into stems of young cuttings (Cloyd, 2015). Adults cause minimal direct plant damage compared to larvae. Nevertheless, both adults and larvae can be vectors of disease by dispersing certain foliar and soil borne phytopathogenic fungi (Cloyd, 2015). An interesting laboratory work from Kühne and Heller (2010) showed that fungus gnats preferably laid their eggs on hyphae of certain phytopathogenic fungi (*Botrytis cinerea*, *Fusarium* species and *Phoma betae*) rather than on other fungi or soil bacteria (*B. pumilus* MCB-7). Even more interesting, fungus gnat larvae did not feed on *B. pumilus* nor affected its survival.

MATERIALS AND METHODS

Series of experiments were performed with various plant models at Laval University greenhouses in 2013, 2016 and 2017 (Table 1). The objective of the trials was to evaluate the effect of *B. pumilus* PTB180 and other strains of *Bacillus* on fungus gnats and western flower thrips infestations when incorporated into growing media. A preliminary trial was conducted in 2013 to investigate the potential insect suppressive effect of various *Bacillus* strains (*B. pumilus* PTB180, *B. subtilis* MBI600, *B. subtilis* PTB185). In this preliminary trial, BioCeres® WP (*Beauveria bassiana*) was used to control insects when infestation levels in the greenhouse were too high.

Ten different plant models were used throughout the trials (*Euphorbia pulcherrima, Brassica oleracea* var. *italica*, *Cucumis sativus*, *Capsicum annuum*, *Zinnia marylandica*, *Begonia semperflorens*, *Salvia* sp., *Cyclamen persicum*, *Petunia* sp., and *Catharanthus roseus*). Insect pest infestations were monitored using sticky traps once a week during five to six weeks. Except for the preliminary trial, there was always a main treatment with the active ingredient *B. pumilus* PTB180, a negative control (PRO-MIX BX®, Premier Tech Horticulture) and a positive control. The main treatment evaluated in all trials was a PRO-MIX BX growing media containing *B. pumilus* PTB180 which was incorporated during manufacturing (PRO-MIX BX® Biofungicide™ + Mycorrhizae™). Mycorrhizae was also present in this growing media, but it is known that this mycorrhiza have no insect suppressive effect (unpublished data, Premier Tech Horticulture). For the positive control, biopesticides available on the market were drenched on the PRO-MIX BX growing media of potted plants during trials (Met52® EC (*Metarhizium anisopliae*) or Vectobac® 600L (*Bacillus thuringiensis* subspecies *israelensis*)) following the manufacturer recommendations. Vectobac was drench on substrate in the second or third weeks following the trials start date.

Table 1. Trials resume.

Trials	Insect pest monitored	Cultivars used	Growing media treatments
2013	Fungus gnats Thrips	Poinsettia	PRO-MIX HP + *B. pumilus* PTB180 PRO-MIX HP + *B. subtilis* PTB185 PRO-MIX HP + *B. subtilis* MBI600 PRO-MIX HP + *B. pumilus* PTB180 + *B. subtilis* PTB185
2013	Fungus gnats	Cyclamen (Halios)	PRO-MIX BX PRO-MIX BX + *B. pumilus* PTB180 PRO-MIX BX + Met52® EC
2016	Fungus gnats	Broccoli (Hyb. BR16) Cucumber (Hyb. Patio snacker) Pepper (Hyb. Mohawk) Begonia (Bada Bing Scarlet)	PRO-MIX BX PRO-MIX BX + *B. pumilus* PTB180 PRO-MIX BX with application of Vectobac® 600L (same treatments for all trials conducted in 2016 and 2017)
Winter 2017	Fungus gnats Thrips	Broccoli (Hyb. BR16) Zinnia (Zahara double Fire) Salvia Begonia (Bada Bing Scarlet)	
Spring 2017	Fungus gnats	Vinca (Hyb. Cora Punch) Pepper (Hyb. Mohawk) Cyclamen Begonia (Bada Bing Scarlet)	
Summer 2017	Fungus gnats	Broccoli (Hyb. BR16) Petunia (Hyb. Supercascade red)	

Greenhouse culture and Infestation method

Plants were seeded in PRO-MIX BX® substrate (Premier Tech Horticulture) and then transplanted in plastic pots containing PRO-MIX substrates corresponding to the growing media treatments. Plants were kept in the greenhouse and maintained in constant growing conditions throughout trial. Greenhouse temperature was maintained at 23°C during the day and 20°C during the night. Natural daylight was supplemented with high-pressure sodium lamps (180 µmol m^{-2} s^{-1}) to keep a 16-h photoperiod. There was no CO_2 enrichment and relative humidity was kept at 40%. All plants received a constant and uniform conventional fertilization within varieties. All plants were grown or placed in the same insect infested greenhouse conditions prior to the trials to let adult fungus gnats and thrips lay their eggs in the substrates. Fungus gnat and thrip population were increased and maintained in the greenhouse by creating favorable conditions for the insect's reproduction (e.g., trays containing growing media with high moisture contents and organic matter were kept in the infested area).

Experimental design

Tests were carried out in isolated cages made of PVC pipes and floating row covers (Agryl P-17, Dubois Agrinovation). Various plant models were tested in each trial. All tests followed a randomized complete block design. There was one cage per treatment and per plant cultivar for each block. Each cage contained six plants of the same cultivar and two sticky traps (Koppert) of the same size to follow the insect infestation evolution. New sticky traps were placed in each cage, seven days before the first insect counts. Almost all trials had four blocks, but the last trial was conducted using 6 blocks to increase the statistical power.

Data collection and processing

Each week, the number of fungus gnats and/or thrips on the sticky traps were counted and noted. Statistical analyses were performed using Dell Statistica software version 12. For each trial, repeated measures ANOVAs were conducted to compare the effect of treatments, plant variety, time and their interactions on the insect infestations. When statistically significant results were found, post-hoc comparisons were carried out using Duncan's test to evaluate the difference between treatments. Log-transformation of data needed to be performed to respect the homoscedasticity and normality assumptions of the tests.

RESULTS AND DISCUSSION

In 2013, we performed the preliminary greenhouse trial on *Euphorbia pulcherrima* (poinsettia) to investigate the potential insect suppressive effect of various *bacillus* strains (*B. pumilus* PTB180, *B. subtilis* MBI600, *B. subtilis* PTB185). Insect suppression effects between *bacillus* strains were not found statistically significant partly due to insufficient statistical power. Nevertheless, we can see in Figure 1 that during infestation peaks, the plants grown in growing media containing *B. pumilus* PTB180 had the lowest number of insects on their sticky cards compared to the other weeks counts. These promising results were sufficient to conduct further trials with *B. pumilus* PTB180.

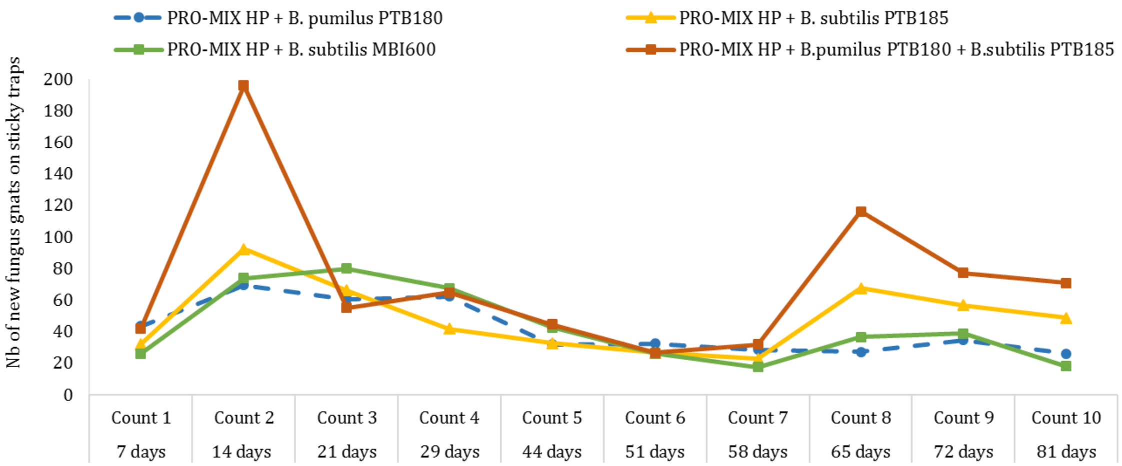

Figure 1. Mean number (*n*=4) of new fungus gnats counted each week on the sticky traps of the different blocks in the greenhouse.

The 2013 trial on cyclamen and the last trial conducted in summer 2017 on petunia and broccoli showed that *B. pumilus* PTB180 incorporated into growing media significantly reduced the number of fungus gnats counted on sticky traps. The results of these trials are described and discussed in the following sub-sections. On the other hand, for the trials against thrips and fungus gnats conducted in 2016, winter 2017 and spring 2017, no statistically significant results were found. Interesting trends were found but infestations variability within treatments was too high to find significant differences. Moreover, in some cases infestations level were extremely low or null which lowered the amount of data collected. The last trial performed in 2017 had less plant varieties tested and more treatments repetitions which allowed us to find statistically significant differences between insect counts on sticky traps.

Efficacy of *B. pumilus* PTB180 against fungus gnats in cyclamen crop

The mean insect counts on sticky traps were always lower in cages were growing media contained *B. pumilus* PTB180 (Figure 2). A repeated measures ANOVA showed that there was a significant effect of growing media treatment on fungus gnats infestation ($F(2,9)=10.11$, $p<0.001$). The post-hoc comparison analysis indicated that the mean number of fungus gnats found on sticky traps was lower for plants grown in substrates containing *B. pumilus* PTB180 (MS=0.342, df=9, p=0.05). There was no significant difference between negative control treatment and the commercial biocontrol treatment. We attribute this to the insufficient number of application of MET52^{mc} in the positive control treatment throughout trial.

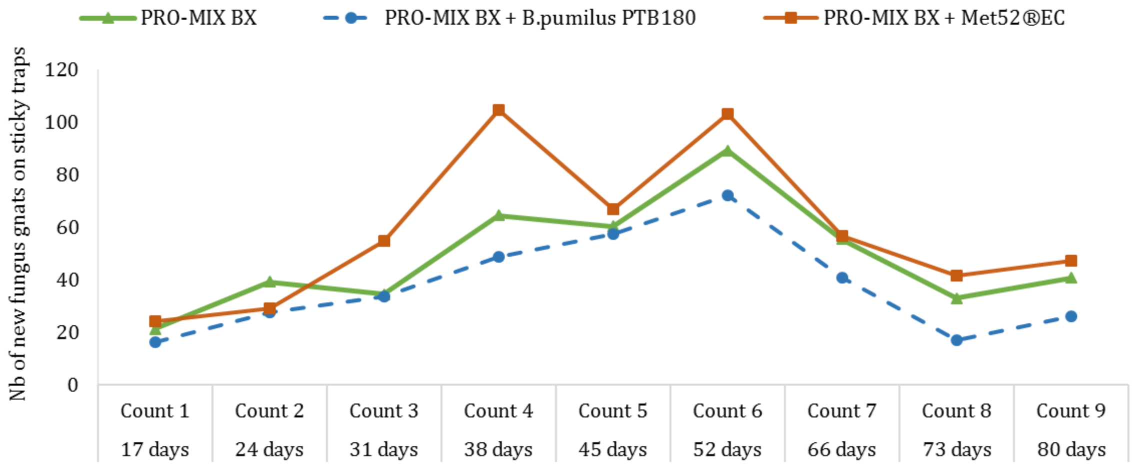

Figure 2. Mean number (n=4) of new fungus gnats counted each week on the sticky traps of the cyclamen cages of the trial performed in 2013.

As observed in the preliminary trial, the infestation peaks were much lower when *B. pumilus* PTB180 was present in the growing media. Furthermore, the infestations levels were reduced from the beginning until 80 days of trial compared to the negative control without the use of other insect control solutions.

Efficacy of *B. pumilus* PTB180 against fungus gnats in petunia and broccoli

The mean insect counts of the treatment with *B. pumilus* PTB180 was lower than the other treatments at all time for both varieties tested (Figure 3). The repeated measures ANOVA showed that the growing media treatments had a significant effect on fungus gnats infestations ($F(2,10)=6.19$, $p=0.017$). The post-hoc comparison analysis indicated that the mean number of fungus gnats found on sticky traps was lower for plants grown in substrates containing *B. pumilus* PTB180 (MS=0.1479, df=10, p=0.05). The repeated measures ANOVA also indicated significant effects of plant variety ($F(1,10)=39.39$, $p<0.001$) and time ($F(4,40)=41.58$, $p<0.001$) on the insect infestation levels. There was no significant interaction between plant variety and growing media treatment. On the other hand, there was a significant interaction between time of count and growing media treatments ($F(8,40)=3.10$, $p=0.008$). The post-hoc comparison analysis results for the time and treatment interaction indicated that the lowering effect of *B. pumilus* PTB180 on insect infestation was observed particularly 7, 14 and 21 days after trial started (MSE=0.0760, df=40, p=0.05).

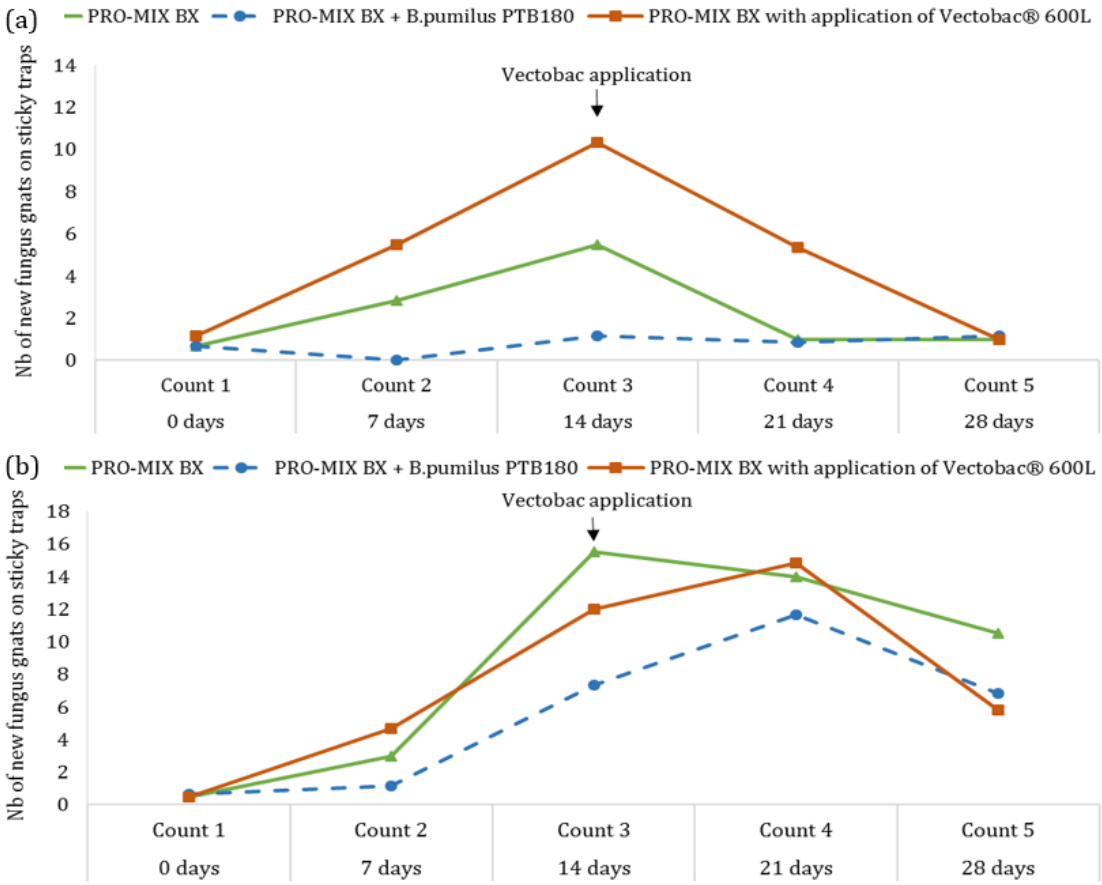

Figure 3. Mean number (*n*=6) of new fungus gnats counted each week on the sticky traps of the (a) petunia cages and (b) broccoli cages of our fourth trial in 2017.

Again, as observed in the previous results, the infestation peaks were much lower or absent when *B. pumilus* PTB180 was present in the growing media. Generally, the infestation levels were lower in this trial than in the previous ones, but the insect suppressive effect of *B. pumilus* PTB180 was still clearly observed during most of the trial. We explain the significant interaction of time of counts and growing media treatments by various peaks of egg hatching. Hence, *B. pumilus* PTB180 treatment had an observable insect suppressive effect when there was egg hatching peaks (count 2, 3 and 4). We hypothesized that *B. pumilus* PTB180 had an effect on the larval stage of fungus gnats and helped to drop the number of adults emerging from the soil. An interesting observation, there was no interaction between treatments and variety. It indicates us that growing media with *B. pumilus* PTB180 should have the same insect suppressive effect whether petunia or broccoli or potentially other varieties of plants are grown.

From our knowledge, no publications have explained the detailed mechanism of *B. pumilus* suppressive effect. Our main hypothesis is that *B. pumilus* PTB180 has a negative effect on fungus gnats' larvae survival by affecting the availability of their preferential food source, phytopathogenic fungi (Braun et al., 2012; Kühne and Heller, 2010). Indeed, *B. pumilus* is already known for its effect against fungal plant pathogens (Leifert et al., 1995; Pérez-García et al., 2011). And some studies have found that fungus gnats prefer to lay their eggs on the mycelium of certain phytopathogenic fungi and suggest that fungus gnat's survival is affected by low fungal abundance (Braun et al., 2012; Kühne and Heller, 2010). Other direct or indirect mechanisms might also be involved. *B. pumilus* PTB180 effect might be due to the activation of the plant defense system producing protease inhibitors. *B. pumilus* PTB180 metabolites, such as lipopeptides, could also be responsible of cell membrane damages (Tozlu et al., 2011). Further research is needed to validate the insect suppression mechanism of *B. pumilus* PTB180.

CONCLUSIONS

In this study, three bacterial strain of *bacillus* were evaluated for their effect of reducing infestation level on two major greenhouse pests, fungus gnats and thrips. Research focus was mainly on the effect of *B. pumilus* PTB180 when incorporated into growing media. *B. pumilus* PTB180 had observable insect suppressive effects against fungus gnats in greenhouse infestation conditions. *B. pumilus* PTB180 have potentially insect suppressive effects against thrips, but further tests and analysis need to be conducted to validate this effect. Considering the results discussed in this article, the use of *B. pumilus* PTB180 as a preventive treatment or as a part of integrated pest management systems present great potential for greenhouse control of fungus gnats and possibly of other flying insects such as thrips. It would be interesting to evaluate if additional applications of *B. pumilus* PTB180 by drench on the substrate surface during high insects infestations would have more important suppressive effect on the insect populations.

This study is still ongoing and further greenhouse trials are being carried out on the effect of *B. pumilus* PTB180 on western flower thrips infestations in horticultural crops at Laval University. Moreover, complementary petri dish tests and greenhouse trials are being conducted, also at Laval University, on the direct effect of the bacterium *B. pumilus* PTB180 and *B. subtilis* PTB185 when applied as a foliar spray for the control of aphids, thrips and fungus gnats.

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