Effect of Plant-growing Media on Western Flower Thrips, *Frankliniella occidentalis*, Pupae and Fungus Gnat, *Bradysia* sp. nr. *coprophila*, Larvae under Laboratory Conditions

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Abstract. Western flower thrips, *Frankliniella occidentalis*, and fungus gnats (*Bradysia* spp.) are major insect pests of greenhouse production systems. Both insect pests have life stages that reside in the soil or plant-growing medium: prepupae and pupae of western flower thrips and fungus gnat larvae. There are unsubstantiated allegations made by a manufacturer that certain plant-growing media that contain a bacterium, *Bacillus pumilus*, and arbuscular mycorrhizal fungus, *Glomus intraradices*, negatively affect the survival of western flower thrips pupae and fungus gnat larvae. Therefore, we conducted a study involving laboratory experiments replicated over time (2019 and 2020) to investigate the influence of the plant-growing media Pro-Mix BX + Mycorrhizae and Pro-Mix BX + Biofungicide + Mycorrhizae on western flower thrips pupae and fungus gnat larvae. All experiments involved placing western flower thrips pupae or fungus gnat larvae (second and third instar) into 473-mL deli containers with the different treatments (plant-growing media). A 5 × 4-cm section of a yellow sticky card was affixed to the lid of each deli container. After 21 days, the number of western flower thrips or fungus gnat adults that emerged from the growing media and were captured on the yellow sticky cards was recorded. The use of the yellow sticky card was an indirect assessment of western flower thrips pupal or fungus gnat larval mortality. We found none of the plant-growing media tested that contained a bacterium and/or arbuscular mycorrhizal fungus affected the survival of western flower thrips pupae or fungus gnat larvae. Therefore, greenhouse producers should be leery of information provided by manufacturers that does not contain valid, scientifically based data.

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), and fungus gnats *Bradysia* spp. (Diptera: Sciaridae) are major insect pests of greenhouse production systems (Cloyd, 2008, 2009; Hamlen and Mead, 1979; Lewis, 1997), with life stages that reside in the soil or plant-growing medium. Western flower thrips pupal life stages (prepupae and pupae) are located in the soil or plant-growing medium (Helyer et al., 1995; Holmes et al., 2012), and the larvae of fungus gnats

reside in the soil or plant-growing medium (Cloyd, 2000; Dennis, 1978; Kennedy, 1974).

The bacterium *Bacillus pumilus* is not considered an insect pathogen like *B. thuringiensis* or *B. sphaericus* (Molina et al., 2010). However, there are unsubstantiated allegations made by a manufacturer that certain plant-growing media containing *B. pumilus* and an arbuscular mycorrhizal fungus, *Glomus intraradices*, negatively affect the survival of western flower thrips pupae and fungus gnat larvae (Bloodnick and Naasz, 2019). Consequently, we conducted a series of replicated experiments to determine whether plant-growing media containing *B. pumilus* and/or *G. intraradices* have a negative effect on western flower thrips pupal and fungus gnat larval survival under laboratory conditions.

Materials and Methods

This study involved two experiments replicated over time to assess the effects of different plant-growing media on western

flower thrips pupae and larval stages of fungus gnats. All experiments were conducted in a laboratory in the Department of Entomology at Kansas State University, Manhattan, KS.

Expt. 1

Western flower thrips. Western flower thrips, *Frankliniella occidentalis*, used in the experiments were maintained under laboratory conditions (24 to 27 °C, 50% to 60% relative humidity, and constant light) in Glad plastic containers [20.4 × 14.4 × 9.4 cm (length × width × height)] (The Glad Products Comp., Oakland, CA). A round hole (diameter, 9.5 cm) was cut in the lid and covered with No-Thrips insect screening (mesh size, 0.15 × 0.15 mm; Greentek, Janesville, WI). Green bean (*Phaseolus vulgaris*) pods purchased from a local supermarket were provided as oviposition sites for adults, and bee pollen (Prairie Harvest; Kansas Wildflower, Wilson, KS) was provided as a food source for adults and larvae. The western flower thrips colony is maintained in a laboratory in the Department of Entomology at Kansas State University, Manhattan, KS.

The experiments investigated the effect of three different plant-growing media on western flower thrips pupae. The three different plant-growing media (treatments) were 1) Berger BM1 (Saint-Modeste, Quebec, Canada), composed of 75% to 85% coarse sphagnum peatmoss, perlite, vermiculite, and a wetting agent (which served as the control); 2) Pro-Mix BX + Mycorrhizae (Premier Horticulture, Inc., Quakertown, PA), composed of 75% to 85% Canadian sphagnum peatmoss, perlite, vermiculite, dolomitic and calcitic limestone, wetting agent, and an arbuscular mycorrhizal fungus (*Glomus intraradices*); and 3) Pro-Mix BX + Biofungicide + Mycorrhizae (Premier Horticulture, Inc.), composed of 75% to 85% Canadian sphagnum peatmoss, perlite, vermiculite, dolomitic and calcitic limestone, wetting agent, a bacterium (*Bacillus pumilus*), and an arbuscular mycorrhizal fungus (*Glomus intraradices*). The experiments were set up as a completely randomized design with three treatments (plant-growing media) and five replications per treatment. The experiment was conducted in 2019 and repeated in 2020.

Using a soft-tipped camel hair brush, 20 western flower thrips pupae were transferred from the colony into a 473-mL deli container with ≈400 mL moist plant-growing medium. The treatments were prepared by mixing 1.6 L tap water into the plant-growing media that were then moistened with an additional 40 mL tap water using a spray bottle before adding western flower thrips pupae. The deli container lids were modified with insect screening (0.2 × 0.8 mm, Greentek) for ventilation, and 12 holes were inserted in the bottom using a dissecting probe, which allowed for any excess liquid to drain or be reabsorbed by the plant-growing medium. In addition, the

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Fig. 1. Mean ($\pm$ SE) number of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards in the 2019 experiment associated with the following treatments: 1) Berger BM1 (control), 2) Pro-Mix BX + Mycorrhizae, and 3) Pro-Mix BX + Biofungicide + Mycorrhizae. Means followed by the same letter are not significantly different ($P > 0.05$) as determined by Fisher's least significant difference test. Vertical bars represent the SE.

holes prevented western flower thrips pupae from drowning or the plant-growing medium from drying out. A 5 × 4-cm section of a yellow sticky card was hot-glued to the lid of each deli container. Each deli container was placed into a plastic petri dish (1.5 × 14 cm) and maintained at 24 to 27 °C under constant light. The deli containers, with the plastic petri dishes, were placed into 30-L Sterilite containers (Sterilite Corp., Townsend, MA) in groups of five (in accordance with each treatment). Afterward, the lids (with six equally spaced 0.5-cm holes) were placed back onto the Sterilite containers. After 21 d, the number of western flower thrips adults captured on each yellow sticky card section was recorded. The use of the yellow sticky card was an indirect assessment of western flower thrips pupal mortality.

Expt. 2

Fungus gnats. The fungus gnat *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae) larvae used in the experiments were maintained as a colony in a laboratory at 24 to 27 °C, 50% to 60% relative humidity, and under constant light in 8-L plastic containers with tight-sealing lids. Openings were cut in the lids (11.5 × 22.5 cm), then insect screening (0.2 × 0.8 mm, Greentek) was hot-glued to the lids to allow for ventilation. Using a 6-

L container, plant-growing medium (Berger BM1; Saint-Modeste) composed of 75% to 85% coarse sphagnum peatmoss, perlite, and vermiculite was moistened with $\approx$ 1.6 L tap water, pasteurized in a microwave (Panasonic Inverter; Panasonic Consumer Electronics Comp., Newark, NJ) at 1250 W for 20 min, and then cooled. The fungus gnat colony is maintained in a laboratory in the Department of Entomology at Kansas State University. Adult specimens used in the study are deposited as voucher numbers 22896–22932 and 32015–32021 in the Illinois Natural History Survey Insect Collection (Champaign-Urbana, IL).

Two tubers (240 g each) of potato (*Solanum tuberosum*) were pureed into small particles, mixed with 125 mL tap water using a food processor, and then mixed uniformly into the plant-growing medium by hand. About 3 L of the growing medium and potato mixture were placed inside the 8-L containers. Sixty grams of oats (*Avena sativa*) (The Quaker Oats Company, Chicago, IL) were placed into two piles positioned in opposite corners of the container on the plant-growing medium surface. The oats were initially moistened with 40 mL tap water using a 946-mL plastic spray bottle (Spraymaster; Delta Industries, King of Prussia, PA). Afterward, the plant-growing medium in the container was moistened daily. About 500 to 1000 adult fungus gnats ($\pm$ 4 d

post-eclosion) were aspirated into a 9-dram (33-mL) plastic vial from the colony and added to the container so that mated females could lay eggs. Fungus gnat adults were added to the container for 2 to 3 d.

To isolate second and third instar fungus gnat larvae, a glass petri dish (100 × 15 mm) was lined with 9-cm-diameter P8 Fisherbrand filter paper (Fisher Scientific, Pittsburgh, PA) and placed into a 750-mL food storage container (Ziploc; SC Johnson, Racine, WI). About 9.5 g of plant-growing medium with pureed potato was placed on top of the filter paper and moistened with a spray bottle, avoiding any standing water. The lid was placed on the storage container and $\approx$ 500 to 1000 adults were collected from the main colony. The adults were released into the containers as described earlier so that mated females could lay eggs. After 11 d, second and early third instar larvae were present for use in the experiments.

The effect of different plant-growing media on second and early third instar fungus gnat larvae of *B. sp. nr. coprophila* was investigated in the experiments. The four different plant-growing media (treatments) were 1) Berger BM1 (Saint-Modeste) composed of 75% to 85% coarse sphagnum peatmoss, perlite, vermiculite, and a wetting agent (which served as the control); 2) Pro-Mix BX + Mycorrhizae (Premier Horticulture, Inc.) composed of 75% to 85% Canadian sphagnum peatmoss, perlite, vermiculite, dolomitic and calcitic limestone, wetting agent, and an arbuscular mycorrhizal fungus (*Glomus intraradices*); 3) Pro-Mix BX + Biofungicide + Mycorrhizae (Premier Horticulture, Inc.) composed of 75% to 85% Canadian sphagnum peatmoss, perlite, vermiculite, dolomitic and calcitic limestone, wetting agent, a bacterium (*Bacillus pumilus*), and an arbuscular mycorrhizal fungus (*Glomus intraradices*); and 4) pasteurized Berger BM1 (Saint-Modeste) composed of 75% to 85% coarse sphagnum peatmoss, perlite, vermiculite, wetting agent, and pureed potato.

We added the pasteurized and pureed potato treatment for the fungus gnat experiment and not the western flower thrips experiment because the western flower thrips pupal stages (prepupae and pupae) do not feed, so no food source was needed. However, fungus gnat larvae do feed, so a treatment similar to our rearing conditions was needed to assess larval mortality associated with no food source. The experiment was set up as a completely randomized design. There were four treatments (plant-growing media) with five replications per treatment. The experiment was conducted in 2019 and repeated in 2020.

Using a glass pipette, 20 second to early third instar fungus gnat larvae were placed into 473-mL deli containers with $\approx$ 300 mL moist plant-growing medium. The pasteurized Berger BM1 with pureed potato treatment was prepared as described. The remaining treatments were prepared by mixing 1.6 L tap water into the plant-growing media, which

Fig. 2. Mean ($\pm$ SE) number of western flower thrips, *Frankliniella occidentalis*, adults captured on yellow sticky cards in the 2020 experiment associated with the following treatments: 1) Berger BM1 (control), 2) Pro-Mix BX + Mycorrhizae, and 3) Pro-Mix BX + Biofungicide + Mycorrhizae. Means followed by the same letter are not significantly different ($P > 0.05$) as determined by Fisher's least significant difference test. Vertical bars represent the SE.

Fig. 3. Mean ($\pm$ SE) number of fungus gnat, *Bradysia* sp. nr. *coprophila*, adults captured on yellow sticky cards in the 2019 experiment associated with the following treatments: 1) Berger BM1 (control 1), 2) Pro-Mix BX + Mycorrhizae, 3) Pro-Mix BX + Biofungicide + Mycorrhizae, and 4) pasteurized Berger BM1 + pureed potato (control 2). Means followed by the same letter are not significantly different ($P > 0.05$) as determined by Fisher's least significant difference test. Vertical bars represent the SE.

were moistened with an additional 40 mL tap water using a plastic spray bottle before adding the fungus gnat larvae. The deli container lids were modified with insect screening (0.2×0.8 mm, Greentek) for ventilation, and 12 holes were inserted in the bottom using a dissecting probe to allow for any excess liquid to drain or be reabsorbed by the plant-growing medium. In addition, the holes prevented fungus gnat larvae from drowning or the plant growing medium from drying out. A 5×4 -cm section of a yellow sticky card was hot-glued to the lid of each deli container. Each deli container was placed into a plastic petri dish (1.5×14 cm) and maintained at 24 to 27 °C under constant light. The deli containers, with the plastic petri dishes, were placed into 30-L Sterilite containers (Sterilite Corp.) in groups of five (in accordance with each treatment). Afterward, the lids (with six equally spaced 0.5-cm holes) were placed back onto the Sterilite containers. After 21 d, the number of fungus gnat adults captured on each yellow sticky card was recorded. The use of the yellow sticky card was an indirect assessment of fungus gnat larval mortality.

Statistical analysis

Data were analyzed using an analysis of variance (ANOVA, $P = 0.05$) (SAS Institute, 2012) with treatment as the main effect. Individual treatment means were separated using Fisher's least significant difference test when the ANOVA indicated a significant treatment effect.

Results and Discussion

There was no significant effect of the three plant-growing media on the mean number of western flower thrips adults captured on the yellow sticky cards in 2019 ($F = 0.83$; $df = 2, 6$; $P = 0.46$) (Fig. 1) or 2020 ($F = 1.16$; $df = 2, 6$; $P = 0.36$) (Fig. 2). However, there was a significant treatment effect associated with the mean number of fungus gnat adults captured on the yellow sticky cards in 2019 ($F = 13.44$; $df = 3, 7$; $P = 0.0004$) (Fig. 3) and 2020 ($F = 9.25$; $df = 3, 7$; $P = 0.0019$) (Fig. 4), with more fungus gnat adults captured on the yellow sticky cards from the pasteurized Berger BM1 + pureed potato treatment. There were no significant differences in the mean number of fungus gnat adults captured on the yellow sticky cards among the Berger BM1 (control), Pro-Mix BX + Mycorrhizae, and Pro-Mix BX + Biofungicide + Mycorrhizae treatments (Figs. 3 and 4).

In conclusion, based on our results, none of the plant-growing media tested that contained a bacterium and/or an arbuscular mycorrhizal fungus negatively affected the survival of western flower thrips pupae or fungus gnat larvae. In addition, it is important that greenhouse producers be cautious in accepting information from manufacturers that does not contain valid, scientifically based data.

Fig. 4. Mean ($\pm$ SE) number of fungus gnat, *Bradysia* sp. nr. *coprophila*, adults captured on yellow sticky cards in the 2020 experiment associated with the following treatments: 1) Berger BM1 (control 1), 2) Pro-Mix BX + Mycorrhizae, 3) Pro-Mix BX + Biofungicide + Mycorrhizae, and 4) pasteurized Berger BM1 + pureed potato (control 2). Means followed by the same letter are not significantly different ($P > 0.05$) as determined by Fisher's least significant difference test. Vertical bars represent the SE.

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