Effect of Diatomaceous Earth and *Trichoderma harzianum* T-22 (Rifai Strain KRL-AG2) on the Fungus Gnat *Bradysia* sp. nr. *coprophila* (Diptera: Sciaridae)

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ABSTRACT This study, consisting of three experiments, was designed to assess whether diatomaceous earth, when applied to the surface of growing media, reduces adult fungus gnat *Bradysia* sp. nr. *coprophila* (Diptera: Sciaridae) emergence or inhibits the females from laying eggs; and whether fungus gnat adults are attracted to the fungus *Trichoderma harzianum* T-22 (Rifai strain KRL-AG2) under laboratory conditions. In the first two experiments, diatomaceous earth was applied at two different thicknesses (3.1 and 6.3 mm) and conditions (dry and moist) to the surface of a growing medium (Universal SB 300 Mix) after the growing medium had been artificially inoculated with second or third instars of fungus gnats, or before female fungus gnat adults were released into each deli squat container. In the third experiment, preparations of the fungus *T. harzianum* at the highest recommended label rate (0.889 kg/m^3) were amended into the growing medium and processed 24, 48, or 72 h before use in a series of three two-choice trials with a two-armed experimental arena. In the first two experiments, the dry or moist layers of diatomaceous earth, in general, did not affect fungus gnats in terms of preventing adult emergence or egg laying by the females. During the course of these experiments, we observed that the diatomaceous earth dry treatments expanded as a result of absorbing moisture from the growing medium, creating fissures that allowed the fungus gnat larvae to pupate and females to lay eggs. In the third experiment, fungus gnat adults were not attracted to the *T. harzianum* treatments in any of the trials.

KEY WORDS pest management, greenhouse, barriers, attraction, fungus gnats

Fungus gnats (*Bradysia* spp.) are major insect pests of greenhouses, particularly under conditions of excessive moisture (Dennis 1978, Hamlen and Mead 1979). Adults typically fly near the growing medium surface, causing minimal direct plant damage, although the females lay eggs that hatch into larvae that reside in the growing medium (Cloyd 2000). The larvae can directly damage plants by feeding on root tissue, thereby disrupting the uptake of water and nutrients (Hungerford 1916, Wilkinson and Daugherty 1970). Fungus gnat larvae also may vector soil-borne pathogens that are responsible for reducing vigor of mature plants (Gardiner et al. 1990, Gillespie and Menzies 1993, Jarvis et al. 1993). Thus, it is important for greenhouse producers to control fungus gnat larvae to avoid economic losses (Leath and Newton 1969, Hamlen and Mead 1979).

The pest management strategy commonly used by greenhouse producers to kill fungus gnat larvae involves applying insecticides to the growing medium (Hamlen and Mead 1979). There are several insecticides used, including conventional larvicides, insect growth regulators, and insecticides derived from bacteria (Lindquist et al. 1985, Cloyd 2000). However, alternative approaches to managing fungus gnats, particularly the larvae, have been discussed among practitioners, and they include incorporating abrasive materials such as diatomaceous earth (DE) into growing media or applying DE to the surface of growing media. Diatomaceous earth is composed of siliceous skeletons of diatoms (Ebeling 1971) that affect insects by removing the cuticular waxes, absorbing oils and waxes in the outer cuticle, and/or rupturing the cuticle causing extensive water loss (Korunic 1998). It has been hypothesized that incorporating DE into soilless growing media will negatively affect fungus gnat adults as they emerge from pupae (Quarles 1992), resulting in increased mortality or a reduction in fitness and reproduction. However, Cloyd and Dickinson (2005) demonstrated that DE, when incorporated into soilless growing media, had minimal affect on the larval stages of the fungus gnat *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae) based on

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adult emergence. This was likely due to the DE losing its abrasive properties under moist conditions (Weinzierl 1998). Furthermore, there is no documentation associated with applying DE to the growing medium surface to either disrupt fungus gnat adult emergence or prevent fungus gnat females from laying eggs. It has even been suggested that a layer of sand placed over the top of the growing medium will reduce infestations of fungus gnats (Hungerford 1916).

Certain fungi serve as a food source for fungus gnat adults (Kennedy 1974, Anas and Reeleder 1988b, Gardiner et al. 1990), and it is possible that adult fungus gnats may be attracted to fungi that reside in growing media. The product RootShield (BioWorks, Fairport, NY), which contains the fungus *Trichoderma harzianum* T-22 (Rifai strain KRL-AG2), is applied as an inoculant to growing media by greenhouse producers to avoid problems with soil-borne fungi, including *Pythium* spp., *Rhizoctonia* spp., *Fusarium* spp., and *Thielaviopsis* spp. (Harman et al. 1989, Harman 2000). However, because fungus gnat adults are attracted to fungi and females tend to lay their eggs near fungal colonies, adult fungus gnats may be attracted to growing media containing *T. harzianum*, which means that plants, developing in a growing medium that has been treated with this fungus, may be exposed to high populations of fungus gnat adults. If females lay eggs in the growing medium, then plants may suffer extensive root damage due to larval feeding.

The purpose of this study was two-fold: first, we wanted to assess whether DE applied to the growing medium surface reduces adult fungus gnat emergence or inhibits females from laying eggs; and second, it was our intention to determine whether fungus gnat adults are attracted to the fungus, *T. harzianum* under laboratory conditions.

Materials and Methods

This study consisted of three laboratory experiments designed to determine the effect of DE and the fungus *T. harzianum* T-22 (Rifai strain KRL-AG2), which is the active ingredient in the product RootShield (BioWorks), on the fungus gnat *Bradysia* sp. nr. *coprophila.*

Experiment 1: Effect of Diatomaceous Earth on the Fungus Gnat, *Bradysia* sp. nr. *coprophila* after Inoculating the Growing Medium with Two Different Instars. This experiment assessed the effect of DE on *B.* sp. nr. *coprophila* when applied to the surface of Universal SB 300 Mix growing medium (SunGro Horticulture; Pine Bluff, AK), after the growing medium had been artificially inoculated with second or third instars. The DE used was produced by Prairierth Farm (Atlanta, IL). The standard procedure for processing 473 ml of deli squat containers (Fabri-Kal Corp., Kalamazoo, MI) was followed as described previously (Cloyd and Dickinson 2006). The deli squat containers were placed into an environmental growth chamber (Sherer Environmental Chamber model CEL-36–10, Warren/Sherer Division of Kysor Industrial Corp., Marshall, WI) set at 24 ± 3°C, 50–60% RH, and a photoperiod of 0:24 (L:D) h for 48 h to allow for the initiation of natural fungal growth (species unknown). Twenty second or third instars of *Bradysia* sp. nr. *coprophila*, obtained from a laboratory-reared colony described in Cabrera et al. (2005), were then applied to the deli squat containers, after which they were returned to the environmental growth chamber for 24 h.

Table 1. Description of treatments, for experiments 1 and 2, involving both dry and moist applications of DE at two thicknesses, and sand to the surface of a growing medium (Universal SB 300 Mix), and an untreated check

Treatment	Description
1	DE applied to surface of growing medium as a 3.1-mm-thick dry layer (by wt: 8.75 g per deli squat container)
2	DE applied to surface of growing medium as a 6.3-mm-thick dry layer (by wt: 17.50 g per deli squat container)
3	DE applied to surface of growing medium as a 3.1-mm moist layer (by wt: 8.75 g per deli squat container followed by an application of 30 ml of deionized water)
4	DE applied to surface of growing medium as a 6.3-mm moist layer (by wt: 17.50 g per deli squat container followed by an application of 30 ml of deionized water)
5	Sand (coarse-quartz) applied to surface of growing medium as a 3.1-mm dry layer (by wt: 52.0 g per deli squat container)
6	Growing medium only

Sixty milliliters of deionized water was applied as a drench to the growing medium inside the deli squat containers immediately before the treatments were placed onto the surface of the growing medium to provide a moist environment ensuring survival of the fungus gnat larvae. A 2.5- by 2.5-cm yellow sticky card (Whitmire Micro-Gen Research Laboratories, St. Louis, MO) was affixed to the underside of the lid of each deli squat container. The purpose of the yellow sticky card was to capture fungus gnat adults as they emerged from the growing medium. There were five replications per instar per treatment resulting in 30 replications per instar, for a total of 60 replications for the entire experiment. The treatments are presented in Table 1. The DE and sand treatments were applied evenly over the surface of the growing medium so that all portions were covered. We only used the 3.1-mm sand thickness because this was almost 3 times what Hungerford (1916) had recommended.

Sixty milliliters of deionized water was applied weekly to a petri dish positioned underneath the deli squat containers for treatments 1, 2, 5, and 6. For treatment 3, 15 ml of deionized water was applied with a 946-ml spray bottle through thrips screening (50 by 24 [0.2 by 0.8 mm]) (Greentek; Edgerton, WI) affixed to the lid of the deli squat containers, and 45 ml of deionized water was applied weekly to the same petri dish located underneath each deli squat container. For treatment 4, 30 ml of deionized water was applied with a 946-ml spray bottle through the thrips screening

affixed to the deli squat container lid, and 30 ml of deionized water was applied weekly to the same petri dish located underneath each deli squat container. Treatments 3 and 4 received different amounts of deionized water to avoid having the surface of the DE dry out. The number of fungus gnat adults captured on the yellow sticky card and flying around inside each deli squat container was counted.

Data were analyzed using a one-way analysis of variance (ANOVA) (SAS Institute 2002). Significant treatment means for the number of fungus gnat adults present in each deli squat container from both second and third instar growing medium samples were separated using a Fisher's protected least significant difference (LSD) test at $P \leq 0.05$. Contrasts were then used to assess the main DE effects of thickness (3.1 and 6.3 mm) and condition (dry and moist), and the interaction in the 2×2 factorial treatment structure.

Experiment 2: Effect of Diatomaceous Earth on the Ability of *Bradysia* sp. nr. *coprophila* Adult Females to Lay Eggs. This experiment followed similar procedures as described for experiment 1, and the same treatments were used (Table 1). However, instead of artificially inoculating the growing medium with 20 fungus gnat second and third instars and then applying the treatments, the treatments were applied to the growing medium surface and then three mated adult female fungus gnats were released into each deli squat container.

A single female and male fungus gnat adult, obtained from the laboratory-reared colony, were aspirated into a 9-dram vial containing Whatman no. 1 (42.5-mm) filter paper (Whatman, Maidstone, England) lightly moistened with deionized water. The fungus gnats were allowed to mate for 24 h. Females were aspirated into another 9-dram vial, three at a time, and then they were released inside each deli squat container, after which the screened lid was immediately secured. Females were allowed to remain in the deli squat container for 48 h, which was sufficient time for each female to lay her compliment of eggs (Meers and Cloyd 2005). After 48 h, the female fungus gnats were removed and a yellow sticky card was affixed to the lid underside of each deli squat container.

The deli squat containers were placed in the same environmental growth chamber as described above for 28 d. The initial procedures used to moisten the growing medium were the same as the first experiment (described above). After 28 d, the number of adult fungus gnats captured on the yellow sticky cards was recorded. Data analysis was the same as for the first experiment described previously.

Experiment 3: Effect of *T. harzianum* T-22 (Rifai strain KRL-AG2) on *Bradysia* sp. nr. *coprophila* Adults. This experiment consisted of four two-choice trials designed to determine whether *T. harzianum* T-22 (Rifai strain KRL-AG2), the active ingredient in RootShield, attracts adult *Bradysia* sp. nr. *coprophila.* All the trials used a set of five two-armed experimental arenas. Each trial was repeated twice, on separate days, with each set of five experimental arenas, such that the number of replicates for each respective trial was 10. Each experimental arena consisted of a central compartment made from a round, clear, 5.3-liter polypropylene microwavable container (Flex & Seal, Rubbermaid, Inc., Fairlawn, OH) with a snap-on lid. Six round holes, 3.8 cm in diameter, were drilled into the sides of the central compartment, equidistant from each other. Every hole was fitted with a cylindrical, acrylic plastic, hollow sleeve (2.8 cm in length with an internal diameter of 3.0 cm). These sleeves were permanently affixed to the central compartment with plastic cement. Hollow, clear acrylic tubes (internal diameter of 2.5 cm) were cut 11.2 cm in length and fitted inside each sleeve. Six smaller compartments, referred to as sample compartments, were attached to the end of each acrylic tube by using the same sleeve as described above. These sample compartments were clear, square, 1-liter polycarbonate microwavable containers (Rubbermaid Stain Shield, Rubbermaid, Inc.) with snap-on lids. For the purpose of this experiment and the subsequent trials, we used two sample compartments that were directly opposite each other whereas the openings of the nonused four sample compartments were sealed with laboratory film (Parafilm, Pechiney Plastic Packaging, Menasha, WI).

Before use in the trials, the glass petri dishes (60 by 15 mm) were placed in an oven set at 100°C for 3 d and at 40°C for 24 h to remove any incidental residues or odors that may possibly confound our results. The petri dishes were rinsed in 95% ethanol and allowed to air dry.

For each trial, ≈100 5-d-old fungus gnat adults (mixture of females and males), obtained from a laboratory-reared colony, were collected into two 9-dram vials (50 fungus gnat adults per vial), and then they were released into the central compartment of the experimental arena. After 48 h, the number of fungus gnat adults captured on the yellow sticky cards in each sample compartment was counted. Data were analyzed, based on the relative number of fungus gnat adults on the yellow sticky cards per treatment, using a *t*-test (SAS Institute 2002).

Trial 1: RootShield versus Deionized Water. This trial compared the attractiveness of RootShield, prepared as a moistened slurry to deionized water. The RootShield treatment was prepared 1 h before initiating the trial by mixing 10 g of RootShield with 12 ml of deionized water and then thoroughly homogenizing the mixture. The resulting moistened slurry was applied with a spoon into each petri dish. A yellow sticky card was then placed directly on the slurry. Twelve milliliters of deionized water was placed into a 60- by 15-mm glass petri dish covered with thrips screening. The thrips screening was attached to the petri dish with silicon. The deionized water was injected through the screening with a needle-tipped syringe. A yellow sticky card was then placed adjacent to the deionized water.

Trials 2–4: Universal SB 300 Mix with RootShield versus Universal SB 300 Mix without RootShield. These three two-choice trials compared the attractiveness of the soilless growing medium (Universal SB 300 Mix)

amended with RootShield to the same soilless growing medium without RootShield. The growing media containing the *T. harzianum* fungus were prepared 24, 48, or 72 h before each trial. There were five replications per treatment. The standard procedure of sterilizing the growing medium in a microwave was used (Cloyd and Dickinson 2006). After the growing medium had cooled, ≈12 g was placed into each 60- by 15-mm petri dish. Treatments containing RootShield were amended with 0.03 g of the product per petri dish (2.37 $in.^3$), which was blended thoroughly to obtain a homogenous mixture. The highest recommended label rate of RootShield (0.889 kg/m^3 of growing medium) was used for all three trials.

The petri dishes containing the growing medium were stored in a small microwave container for 24 h, after which they were placed inside the designated sample compartments. A yellow sticky card was placed directly on the surface of each RootShield preparation and growing medium only treatment.

For the 48- and 72-h preparations of RootShield, all petri dishes were sealed with laboratory film (Parafilm, Pechiney Plastic Packaging) and placed into an environmental chamber (described above) with a photoperiod of 0:24 (L:D) h for 48 and 72 h before use in the appropriate trials.

Gravimetric moisture content of each growing medium sample was determined both before and after conducting the trials based on five samples placed into petri dishes (60 by 15 mm) and then drying the growing medium to a constant mass in a forced air drying oven at 60 ± 1°C. This established the mean moisture content for each growing medium expressed as a percentage. The formula used to obtain the percentage of moisture content was as follows:

$$\% \text{ moisture content} = (B - A) - (C - A)/(B - A) \times 100$$

where A is weight (grams) of petri dish, B is initial weight (grams) (petri dish + moist growing medium), and C is final weight (grams) (petri dish + dry growing medium).

Data were analyzed, based on the percentage of moisture content per treatment, by using a *t*-test (SAS Institute 2002).

Results

Experiment 1: Effect of Diatomaceous Earth on the Fungus Gnat, *Bradysia* sp. nr. *coprophila* after Inoculating the Growing Medium with Two Different Instars. There were no significant differences among the treatments, based on the number of fungus gnat adults recovered on the yellow sticky cards and flying, for the deli squat containers inoculated with fungus gnat second instars ($F = 1.04$; df = 5, 29; $P = 0.415$) or third instars ($F = 1.96$; df = 5, 29; $P = 0.121$). The number of fungus gnat adults that emerged from each of the DE treatments including thickness (3.1 and 6.3 mm) and condition (dry and moist), and the sand treatment (3.1 mm in thickness), was not significantly different from the control or growing medium-only treatment (Table 2).

Table 2. Mean ± SE number of *Bradysia* sp. nr. *coprophila* adults that emerged from 473-ml deli squat containers with the growing medium covered with either a dry or moist layer (3.1 and 6.3 mm in thickness, respectively) of DE and a 3.1-mm layer of sand

Treatment	Second instar	Third instar
1. DE 3.1-mm layer (dry) on GM[z]	18.2 ± 0.84a	17.8 ± 0.75a
2. DE 6.3-mm layer (dry) on GM	17.6 ± 0.84a	17.4 ± 0.75a
3. DE 3.1-mm layer (moist) on GM	17.0 ± 0.84a	16.2 ± 0.75a
4. DE 6.3-mm layer (moist) on GM	17.0 ± 0.84a	15.0 ± 0.75a
5. Sand 3.1-mm layer (dry) on GM	17.2 ± 0.84a	17.2 ± 0.75a
6. Control (GM only)	15.6 ± 0.84a	16.0 ± 0.75a

The deli squat containers were initially inoculated with 20 second or third instars for all six treatments. There were five replications per instar per treatment. Means within a column followed by a common letter are not significantly different ($P = 0.05$) as determined by Fisher's protected LSD test.

[z] GM, growing medium (Universal SB 300 Mix, SunGro Horticulture).

The main DE effects of thickness (3.1 and 6.3 mm) and condition (dry and moist) were not significantly different for the second instars (depth, $P = 0.72$; condition, $P = 0.72$) and third instars (depth, $P = 0.29$; condition, $P = 0.29$) based on the 2 × 2 factorial analysis. In addition, the interaction was not significant for either instar (second instar, $P = 0.72$; third instar, $P = 0.59$).

Experiment 2: Effect of Diatomaceous Earth on the Ability of *Bradysia* sp. nr. *coprophila* Adult Females to Lay Eggs. There was a significant difference among the treatments in the number of fungus gnat adults recovered on the yellow sticky cards ($F = 4.06$; df = 5, 29; $P = 0.0083$) with both the dry DE treatments (3.1 and 6.3 mm in thickness) having significantly fewer adult fungus gnats emerging than the sand and control (growing medium-only) treatments (Table 3). However, none of the DE treatments, dry or moist, were significantly different from each other. Furthermore, both the moist DE treatments, at 3.1 and 6.3 mm in

Table 3. Mean ± SE number of *Bradysia* sp. nr. *coprophila* adults that emerged from 473-ml deli squat containers with the growing medium covered with either a dry or moist layer (3.1 and 6.3 mm in thickness, respectively) of DE, or a 3.1-mm layer of sand for all six treatments

Treatment	No. fungus gnat adults that emerged
1. DE 3.1-mm layer (dry) on GM[z]	40.2 ± 6.5b
2. DE 6.3-mm layer (dry) on GM	29.4 ± 6.5b
3. DE 3.1-mm layer (moist) on GM	47.6 ± 6.5ab
4. DE 6.3-mm layer (moist) on GM	47.2 ± 6.5ab
5. Sand 3.1-mm layer (dry) on GM	65.6 ± 6.5a
6. Control (GM only)	59.8 ± 6.5a

Three mated adult females were placed into the deli squat containers after treatments had been applied and allowed to lay eggs for 48 h. There were five replications per treatment. Means within a column followed by a common letter are not significantly different ($P = 0.05$) as determined by Fisher's protected LSD test.

[z] GM, growing medium (Universal SB 300 Mix, SunGro Horticulture).

Table 4. Mean ± SE number of *Bradysia* sp. nr. *coprophila* adults captured on yellow sticky cards (2.5 by 2.5 cm) for each treatment within a trial when comparing a water slurry of the fungus *T. harzianum* T-22 (Rifai strain KRL-AG2) (RootShield) with deionized water (trial 1), and three treatments using growing medium (Universal SB 300 Mix) containing the fungus prepared either 24, 48, or 72 h beforehand (trials 2–4)

Trial	Treatment	No. fungus gnat adults captured			
		RootShield[a]	MC[b]	No RootShield	MC
1	Water	36.8 ± 4.4a	—	48.5 ± 5.3a	—
2	24 h	51.5 ± 3.8a	75A	61.0 ± 7.8a	73A
3	48 h	55.7 ± 4.2a	66A	49.7 ± 5.6a	66A
4	72 h	34.6 ± 7.1a	62A	38.0 ± 5.8a	63A

All comparisons with the exception of trial 1 involved the growing medium inoculated with RootShield vs. a noninoculated growing medium. There were 10 replications per treatment. Treatment means within a row followed by a common lower case letter are not significantly different ($P = 0.05$) as determined by *t*-test. Treatment means within a row followed by a common upper case letter are not significantly different ($P = 0.05$) as determined by *t*-test.

[a] RootShield rate was 1.5 lb/y^3 of growing medium or 2.37 in.^{3} (0.03 g) per petri dish.

[b] MC, percentage of moisture content.

thickness, were not significantly different from the sand or control treatments (Table 3).

The main DE effects of thickness (3.1 and 6.3 mm) and condition (dry and moist) were not significantly different ($P = 0.39$ and $P = 0.06$, respectively) based on the 2 × 2 factorial analysis. In addition, the interaction was not significant ($P = 0.43$).

Experiment 3: Effect of *T. harzianum* T-22 (Rifai strain KRL-AG2) on *Bradysia* sp. nr. *coprophila* Adults. The results from the four trials are presented in Table 4. None of the treatments were significantly different from each other in all four trials (trial 1: $F = 1.44$; df = 9, 18; $P = 0.111$; trial 2: $F = 4.13$; df = 9, 18; $P = 0.294$); trial 3: $F = 1.72$; df = 9, 18; $P = 0.407$; trial 4: $F = 1.50$; df = 9, 18; $P = 0.715$) based on the number of fungus gnat adults captured on yellow sticky cards per treatment (Table 4). The percentage of moisture content of the growing media, between the treatments, in the three trials with growing medium (2–4) was not significantly different after 24 h ($F = 5.48$; df = 9, 18; $P = 0.38$), 48 h ($F = 1.45$; df = 9, 18; $P = 0.70$), and 72 h ($F = 25.97$; df = 9, 18; $P = 0.65$) (Table 4).

Discussion

The dry and moist DE and sand treatments had no affect on fungus gnats in terms of inhibiting adult emergence or preventing egg laying by females. Adsorption of moisture by DE particles reduces the abrasive properties and thus effectiveness against arthropod pests that reside in the growing medium (Maceljski and Korunic 1971, Weinzierl 1998). In addition, during the course of the first two experiments we observed that the DE dry treatments expanded, providing fissures (or openings) that allowed the fungus gnats to pupate and females to lay eggs. Hungerford (1916) indicated that a 1.27-mm dry sand layer placed over the top of a growing medium inhibits fungus gnats. It was suggested that fungus gnat larvae may have difficulty migrating through the dry sand barrier. However, we found that a 3.1-mm layer of sand had no affect on either preventing adult fungus gnat emergence or on inhibiting females from laying eggs. The use of DE or sand as a barrier, applied to growing media, may not be a feasible option, because plants would have to be watered only using subirrigation systems since overhead watering would disrupt the DE or sand barriers, thereby nullifying their use. In addition, DE absorbs moisture from the growing medium, resulting in fissures developing, which, as we noted, allowed fungus gnats to pupate and females to lay eggs. For example, although both the dry DE treatments (Table 1, treatments 1 and 2), at 3.1 and 6.3 mm in thickness, were initially considered dry, any moisture present in the growing medium would slowly migrate through the dry DE, increasing its wetness. Again, this resulted in fissures developing, so there was no longer a uniform layer of DE on the growing medium surface. This also occurred in the 6.3-mm dry DE layer, which had the fewest mean number of fungus gnat adults emerge, although the 6.3-mm dry DE layer treatment was only significantly different from the sand and control treatments; not the other DE treatments (Table 3).

In the second experiment, we observed, after releasing the female fungus gnats that occasionally they would get stuck in both of the moist DE treatments (Table 1, treatments 3 and 4). However, the data from this experiment indicate that at least one female fungus gnat adult survived, when applied to the deli squat containers, and she laid eggs, because all the replications had adults emerge. The number of fungus gnat adults that emerged per deli squat container is similar to the number of eggs laid by a single female over a 48-h period (Meers and Cloyd 2005). The moist DE treatments (both 3.1 and 6.3 mm in thickness) formed bubbles on the surface, which eventually resulted in holes that provided openings for the fungus gnat larvae to pupate. This may have allowed the adults to avoid the DE layer when emerging. We also observed fungus gnat pupae protruding through both the sand and DE covering, indicating that the fourth instars were not inhibited by these barriers. Pupal cases were even visible through the surface of the DE in areas not associated with the crevices created as the material expanded.

Although fungus gnat larvae and adults will feed on certain fungi as a food source (Kennedy 1974; Anas and Reeleder 1988a, 1988b; Kuhne and Muller 1996), in our study, the fungus, *T. harzianum* strain T-22 (KRL-AG2), the active ingredient in the product RootShield, was not attractive to fungus gnat adults at the concentrations tested. It is possible that this fungus is not a viable food source for survival. For example, Anas and Reeleder (1988b) reported that larvae of the fungus gnat *Bradysia coprophila* Lintner (Diptera: Sciaridae) failed to use a related species, *Trichoderma viride*, as a food source. This suggests that greenhouse producers should be able to use this product on their crops without having to be concerned with luring

fungus gnat adults and possibly suffering plant damage due to larval feeding. However, it may be that the adults are attracted to the secondary metabolites produced by fungi, providing an indication that the substrate is suitable for larval development (Frank and Dettner 2001) or that it is a viable food source. Although *T. harzianum* does produce volatile constituents (Zeid et al. 1999, Zeid and El-Esnawy 2003, Jannet et al. 2005), these volatiles may not be detected by fungus gnat adults. Further studies need to be conducted to assess whether fungus gnats will feed on this fungus and if so, to determine the affect on development and reproduction. The moisture content of the growing medium, with and without *T. harzianum*, were similar (Table 4), which likely removed the possibility that moisture content was responsible for the results obtained although, overall, there were no significant differences between any of the treatments.

This study has demonstrated that applications of DE (dry or moist) or sand, applied at varying thicknesses (3.1 and 6.3 mm), to the surface of growing media fail to inhibit adult emergence or disrupt females from laying eggs, which suggests that applying barriers such as DE, sand, or a combination may not be an effective pest management strategy for fungus gnats in greenhouses or interiorscapes. In addition, the fungus, *T. harzianum* strain T-22 (KRL-AG2) in the product RootShield does not attract fungus gnat adults, indicating that greenhouse producers may not promote fungus gnat problems when using this product.

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