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Effects of Growing Media Containing Diatomaceous Earth on the Fungus Gnat *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae)

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Abstract. **Fungus gnats (*Bradysia* spp.) are major insect pests in greenhouses. The adult stage is primarily a nuisance whereas the larval stage is directly responsible for plant injury by feeding on plant roots or tunneling into stems. Insecticides are used to deal with fungus gnat larvae in growing medium, although sometimes with limited success. This study evaluated the potential of using a soil amendment—diatomaceous earth (DE) incorporated into growing media—for controlling the fungus gnat *Bradysia* sp. nr. *coprophila*. Two experiments were conducted by testing a series of growing media containing various concentrations of diatomaceous earth, and several without diatomaceous earth. The effects of the growing media containing diatomaceous earth on both the 2nd and 3rd instars of fungus gnat larvae were determined by recording the number of adults captured on yellow sticky cards (2.5 × 2.5 cm). Based on the results obtained from both experiments, the addition of DE to growing medium, at the concentrations tested, did not negatively affect or increase efficacy against both the 2nd and 3rd instars. This suggests that incorporating DE into commercially available growing medium may not be beneficial to greenhouse producers. However, further research is needed to assess whether differential larval susceptibility and moisture content influence the ability of DE to control soil-dwelling arthropods.**

Fungus gnats (*Bradysia* spp.) (Diptera: Sciaridae) are common insect pests in greenhouse production systems (Dennis, 1978; Hamlen and Mead, 1979), particularly during propagation, which provides an ideal environment for population growth (Cloyd, 2000). The primary damaging stage is the larva, which feed on plant roots disrupting their ability to uptake water and nutrients (Hungerford, 1916; Leath and Newton, 1969; Wilkinson and Daugherty, 1970). In addition, larva can vector soilborne pathogens directly through feeding or creating wounds that allow entry for soilborne pathogens (Gardiner et al., 1990; Jarvis et al., 1993; Gillespie and Menzies, 1993).

Greenhouse producers traditionally use insecticides to control fungus gnat larvae (Hamlen and Mead, 1979; Lindquist et al., 1985). A conventional larvicide or insect growth regulator, applied as a drench, is generally successful in controlling the larval stage (Lindquist, 1994). However, due to regulatory restrictions on the use of insecticides (Sray, 1997) and the potential for resistance as a result of continual reliance on insecticides (Lacey and Mulla 1977; Nedstam and Burman, 1990) greenhouse producers are seeking more long-term alternative management strategies that will alleviate problems with fungus gnats.

An alternative management strategy may be the use of growing media that contain amendments such as diatomaceous earth. Diatomaceous earth (DE) is composed of the siliceous skeletons of diatoms (Ebeling, 1971). Diatomaceous earth acts by removing the insect's cuticular waxes and by absorbing oils and waxes in the outer cuticle (Ebeling, 1971). Another way in which DE may kill insects is through desiccation—that is by rupturing or abrading the insect cuticle causing extensive water loss (Korunic, 1998). Insects pick up DE particles on their cuticle as they move (Le Patourel et al. 1989). However, the origin of the material and physical characteristics can affect insecticidal properties (Korunic, 1998). The primary use of DE in pest management programs has been for control of stored product pests (Arthur, 2000a, 2000b; Quarles and Winn, 1996). Diatomaceous earth has been shown to be an effective alternative to pesticides for control of several stored product pests such as *Tribolium castaneum* Herbst (Rigaux et al., 2001), *Tribolium confusum* Jacquelin du Val, *Tenebrio molitor* L., *Sitophilus granarius* L., and *Plodia interpunctella* Hübner (Mewis and Ulrichs, 2001), and *Cryptolestes ferrugineous* Stephens (Korunic et al., 1996).

It has been hypothesized that incorporating DE into soil will control insects emerging from pupal stages (Quarles, 1992). However, there has been little, if any, research conducted to quantitatively demonstrate the use of growing media containing DE for control of soilborne insect pests such as fungus gnats, *Bradysia* spp. As a result, the purpose of this study was to determine if growing media containing different concentrations of DE negatively affect the fungus gnat *Bradysia* sp. nr. *coprophila* (Lintner).

Materials and Methods

This study, which consisted of two similar experiments, was conducted in the National Soybean Research Laboratory at the University of Illinois, Urbana-Champaign. Growing media were acquired from Sun Gro Horticulture (Marysville, Ohio) on 14 Feb. 2004. The growing media used in the study were Sunshine LC1 Mix, SB300 Universal, and Teufel Mix. Sunshine LC1 Mix was the base-growing medium in which DE was added to obtain the desired formulations. The components of this growing medium include peat moss, perlite, lime, a fertilizer charge, and a wetting agent. The growing media SB300 Universal and Teufel Mix, in addition to the components found in Sunshine LC1 Mix, also contain bark and vermiculite. Both these growing media did not contain DE. The DE formulations used in the study were Diafil (World Minerals, Inc., Santa Barbara, Calif.), Dicalite (Grefco, Lompoc, Calif.), and Fine Perlite (Seba Beach, Canada).

Fungus gnats. Fungus gnats used in this study were obtained from a laboratory colony of *Bradysia* sp. nr. *coprophila* (Lintner) maintained in moist soilless growing medium supplemented with shredded potato and oatmeal (Cloyd and Zaborski, 2004).

Experimental procedures. Fungus gnat larvae were reared to a known age using the following procedure. A standard glass petri dish (100 × 20-mm) was lined with moistened 90-mm Whatman No. 1 filter paper (Whatman, Maidstone, U.K.). The petri dish was filled with a mixture of sterilized Universal Mix (pine bark compost, Canadian sphagnum peat, horticultural vermiculite, perlite, and a wetting agent) and pureed potatoes at a ratio of 6 parts growing medium to 1-part potatoes. About 0.85 g of rolled oatmeal was sprinkled onto the surface and then the growing medium was moistened with 35-mL of deionized water using a 946-mL spray bottle. The petri dish was enclosed in a 739-mL Ziploc container with ventilation holes. About 30 to 40 fungus gnat adults (mixture of female and male) were collected from a laboratory colony into a 9-dram plastic vial (BioQuip Products, Rancho Dominguez, Calif.) secured with a cap, and then the vial (with the cap removed) was enclosed inside a Ziploc container, which was then placed into an environmental growth chamber (model CEL-36-10; Warren/Sherer Division of Kysor Industrial Corp., Marshall, Mich.) at a temperature of 24 ± 3 °C. The petri dish (with the cap removed) remained in the chamber for 48 h to allow the female to mate, and then lay eggs. After 48 h, the petri dish was removed from the Ziploc container and 1.0 mL of deionized water was applied to the growing

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medium surface. A glass lid was placed on the petri dish, which was then returned to the growth chamber. The petri dish was checked daily and 0.5 mL of deionized water was applied to the surface to prevent the growing medium from drying out. Under the environmental conditions of the growth chamber, fungus gnat larvae were 2nd instars after 7 to 8 d, and 3rd instars after 10 to 11 d using size to differentiate between the larval instars (Zaborski and Cloyd, 2004).

The petri dish surface was carefully evaluated using a dissecting microscope, to assess the fungus gnat larval population. A small sample of growing medium (0.85 to 1.4 g) containing larvae was removed, using a laboratory spoon, from the original petri dish and placed into another glass petri dish (100 × 20 mm). The sample was carefully washed with deionized water and then the petri dish was filled with water. The petri dish was examined using a dissecting microscope and any floating larvae were collected with a micropipette. The larvae were placed into a small glass petri dish (60 × 15 mm) and covered with deionized water. Larvae remained in the water for up to 30 min or until they were applied to the samples. Before inoculating the samples, 2nd and 3rd instars were counted again using a dissecting microscope to double check counts.

The growing media tested and used for the samples were placed into a dishpan and moistened with deionized water. Each growing medium sample was thoroughly mixed 48 h before adding fungus gnat larvae. The sample consisted of 300 mL of growing medium. The growing medium was measured into a 600-mL glass beaker and compressed (to remove air space) to the 300-mL mark. In total, 1.4 g of rolled oatmeal was applied to the sample and 100 mL of deionized water was added, except for the Teufel mix in which 85 mL of deionized water was added. The sample was mixed and then placed into a 473-mL polypropylene deli container (Fabri-Kal Corp.; Kalamazoo, Mich.) and compressed again. Ten to twelve small holes (about 2 mm) were punctured on the bottom using a dissecting probe. The samples were placed into the growth chamber for 48 h, which allowed time for fungal growth, before inoculating with larvae. Both 2nd and 3rd instars were used. Seven-day-old 2nd instars and 11-d-old 3rd instars were applied to the growing medium samples. Twenty larvae (2nd or 3rd instar depending on the sample) from the petri dish (described above) were poured onto each sample and then the petri dish was rinsed with 50 mL of deionized water to ensure that all larvae had been placed onto the growing medium. There were seven replications per sample (n = 12) for each larval instar (n = 2) for a total of 168 samples. The inoculated samples were then returned to the growth chamber. Each deli container was placed onto the lid of a petri dish (100 × 20 mm) containing water, which could be taken up through the holes on the bottom of the deli containers. This prevented the growing medium from drying out. Every week, 50 mL of deionized water was added to the petri dish lids to maintain a consistent moisture level. In addition, 4.0 mL of deionized water was applied to the surface of the growing medium every week. Each deli container had a yellow sticky card (2.5 × 2.5 cm) attached to the underside of the lid to capture adults that emerged from the growing medium. Both experiments were set-up as a completely randomized design.

Data for the number of adults captured on the yellow sticky cards as well as the number of adults that were flying around within the deli container for each experiment were analyzed using a one-way analysis of variance (ANOVA) (SAS Institute, 2001) with growing medium as the main effect. The data were normally distributed for the adult counts. The means for the number of fungus gnat adults recovered from both 2nd and 3rd instar samples for the different growing media were separated using a Fisher's protected least significant difference (LSD) test at $P \leq 0.05$.

Gravimetric moisture content of each growing medium sample was determined both before and after conducting the experiments based on five samples placed in petri dishes (100 × 20 mm) and then drying 100 mL of 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 percent moisture content was

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

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

Results

Experiment 1. Percent moisture content before the experiment ranged from 24% to 66% (Table 1). Percent moisture content after the experiment ranged from 49% to 82% for the growing media inoculated with 2nd instars and 53% to 76% for growing media inoculated with 3rd instars. The moisture content for the SB300 Universal was always much lower than the other growing media (24%, 49%, and 53%, respectively) (Table 1). Growing medium effect was significant for the number of fungus gnat adults recovered from samples inoculated with 2nd instar larvae ($F = 2.57$; df = 11, 83; $P = 0.0083$) with the growing medium containing the highest concentration of DE in the Dicalite formulation (30 lb DE/yard^3) having the lowest adult emergence (5.4 ± 0.5; mean ± SE) (Table 2). This growing medium was significantly different from all the other growing media tested with the exception of the Sunshine LC1 Mix (6.0 ± 0.8; mean ± SE), SB300 Universal (8.4 ± 1.6; mean ± SE), and the lowest concentration of DE in the formulation Diafil (10 lb DE/yard^3) (6.5 ± 1.4; mean ± SE). Growing medium was not significant for the number of fungus gnat adults recovered from samples inoculated with

Table 1. Percent moisture content of final material (growing media) containing diatomaceous earth (DE) tested before and after the first experiment.

			Moisture content (%)		
	Concn		Before	After	
Formulation name	(lb DE/yard^3)	n	2nd and 3rd Instar	2nd Instar	3rd Instar
Diafil	10	5	53	78	75
Diafil	20	5	52	76	71
Diafil	30	5	53	76	68
Dicalite	10	5	53	76	69
Dicalite	20	5	52	80	71
Dicalite	30	5	53	74	65
Fine Perlite	10	5	61	78	75
Fine Perlite	20	5	56	77	73
Fine Perlite	30	5	53	76	65
Teufel Mix	---	5	50	62	57
Sunshine LC1 Mix	---	5	66	82	76
SB300 Universal	---	5	24	49	53
Mean (± SE)			52.1 ± 2.9	73.6 ± 2.6	68.1 ± 2.1
Range			24–66	49–82	53–76

Table 2. Mean adult fungus gnat (*Bradysia* sp. nr. *coprophila*) emergence based on yellow sticky card (2.5 × 2.5 cm) counts from growing medium samples initially inoculated with 2nd and 3rd instar fungus gnat larvae for final material (growing media) containing diatomaceous earth (DE) and other growing media tested for the first experiment. There were about 20 fungus gnat larvae used per replication.

Formulation name	n	Concn (lb DE/yard^3)	2nd Instar (mean ± SE)	3rd Instar (mean ± SE)
Diafil	7	10	6.5 ± 1.4 bcd[z]	11.5 ± 1.4 a
Diafil	7	20	10.0 ± 1.2 a	10.7 ± 1.7 a
Diafil	7	30	9.0 ± 1.0 abc	11.3 ± 0.8 a
Dicalite	7	10	10.8 ± 0.8 a	11.6 ± 1.2 a
Dicalite	7	20	10.0 ± 0.5 a	12.6 ± 0.8 a
Dicalite	7	30	5.4 ± 0.5 d	8.4 ± 1.0 a
Fine Perlite	7	10	9.5 ± 0.8 ab	12.3 ± 1.2 a
Fine Perlite	7	20	9.4 ± 1.6 ab	11.1 ± 0.4 a
Fine Perlite	7	30	9.4 ± 0.6 ab	12.6 ± 1.1 a
Teufel Mix	7	---	9.7 ± 0.9 a	7.8 ± 1.1 a
Sunshine LC1 Mix	7	---	6.0 ± 0.8 cd	11.4 ± 0.9 a
SB300 Universal	7	---	8.4 ± 1.6 abcd	11.3 ± 1.3 a

[z]Means not followed by a common letter are significantly different ($P = 0.05$) as determined by Fisher's protected least significant difference (LSD) test.

3rd instar larvae (F = 1.63; df = 11, 83; P = 0.109) (Table 2).

Experiment 2. Percent moisture content before the experiment ranged from 43% to 66% (Table 3). After the experiment, percent moisture content ranged from 71% to 85% for the growing media inoculated with 2nd instars and 75% to 85% for growing media inoculated with 3rd instars. In contrast with the first experiment, the moisture content for the SB300 Universal was only lower (43%) before the experiment was conducted (Table 3). Growing medium was not significant for the number of fungus gnat adults recovered from samples inoculated with 2nd instars (F = 1.69; df = 11, 83; P = 0.095), however, growing medium was significant for the number of fungus gnat adults recovered from samples inoculated with 3rd instars (F = 3.36; df = 11, 83; P = 0.001) with all the growing media having lower adult emergence values than SB300 Universal (Table 4).

Discussion

The insecticidal activity of DE may depend on a number of factors such as uniform particle size (≥10 μm), percent of particles with a diameter <12 μm, distribution of diatom particles, and oil adsorption capacity (Korunic 1998). However, insect sensitivity to DE may be related more to anatomy and physiology. For example, insects with a large surface area in relation to volume of body, rough or hairy body surface, and thin cuticle thickness are more sensitive to DE, which may be related to larval instar stage or adult (Carlson and Ball, 1962). In fact, there may be a wide variation in insect susceptibility to DE (Rigaux et al., 2001).

Any variation in larval susceptibility such as the 2nd and 3rd instars of fungus gnat to DE may be due to reduced movement, cuticle thickness (Korunic, 1998), and where fungus gnats pupate (Zaborski and Cloyd, unpublished data). Insects that are active are more likely to be damaged than sedentary insects (Fields and Korunic, 1996). Minimal movement by the larval stage in the growing medium could result in less DE, depending on concentration, coming in contact with the insect's body (cuticle) as it migrates through the growing medium profile (Rigaux et al., 2001). Diatomaceous earth will affect insects as long as there is a sufficient concentration to ensure that insects come in contact with enough diatom particles (Korunic 1998). Any variation in the concentration of DE may impact efficacy as insects are exposed to fewer diatom particles as less DE is incorporated into the growing medium. However, it has not been shown that one instar of fungus gnat is more active than the other or there are differences in larval stage susceptibility. In the first experiment, growing media (those with and without DE) appeared to have a numerically greater negative effect, based on adult emergence, for 2nd instars compared to 3rd instars as more adults (on average) tended to emerge from growing media inoculated with 3rd instars than 2nd instars (Table 2). It is possible this is due to the 3rd instars having a thicker cuticle, which could decrease their susceptibility to injury from either DE or other growing medium particulates, resulting in less mortality. The one exception to this hypothesis is the Teufel mix in which fewer adults emerged in the 3rd instar inoculated growing medium than the 2nd instar inoculated growing medium. Additionally, the location of fungus gnat larvae in the growing medium profile may influence the efficacy of DE. Preliminary studies have demonstrated that fungus gnat larvae and pupae are distributed throughout the growing medium profile (Zaborski and Cloyd, unpublished data), which may influence susceptibility to growing media containing lower concentrations of DE. Also, fungus gnat larvae feeding within plant roots or stems may escape any detrimental affects from growing media containing DE (Hungerford, 1916). It has been suggested that fungus gnat adults may be negatively affected by growing medium containing DE as they emerge from pupae (Quarles, 1992) resulting in increased mortality and/or reduced fitness and reproduction. The reason why there were no significant differences among the growing media for the 3rd instars was due to the low adult emergence from all the growing media (Table 4).

In general, the percent moisture content of the growing media used before each experiment were similar based on the mean (± SE) moisture content (52.1 ± 2.9 for the first experiment and 51.2 ± 1.8 for the second experiment) and range of moisture contents (24% to 66% for the first experiment and 43% to 66% for the second experiment) (Tables 1 and 3) with the exception of SB300 Universal (24% vs. 43%, respectively). The variable moisture content of the SB300 Universal may be due to the physical characteristics or composition of the components.

The one noticeable difference in the data, based on recovery rate, was the lower number of fungus gnat adults obtained from 3rd instars in the second experiment compared to the first experiment (Tables 2 and 4). This may be a response to the different percent moisture contents between both experiments. Measurements of percent moisture content did vary after completion of the experiments in regards to both instars with percent moisture contents (mean ± SE) for the 2nd and 3rd instars in experiment one lower (73.6 ± 2.6 and 68.1 ± 2.1, respectively) than those for the 2nd and 3rd instars in the second experiment (81.5 ± 1.2 and 80.3 ± 1.6, respectively) (Tables 1 and 3). These differences in percent moisture content may account for the variability in recovery rates experienced in both experiments for the 3rd instars. Although the range of fungus gnat adult emergence was similar for 2nd instars in both experiments (5.4 to 10.8 and 5.1 to 10.4, respectively), the relative number of adults that emerged was lower in the second experiment compared to the first experiment (Tables 2 and 4). Again, this may be due to

Table 3. Percent moisture content of growing media (formulation name) containing diatomaceous earth (DE) tested before and after the second experiment.

Formulation name	Concn (lb DE/yard^3)	n	Moisture content (%) Before	After	
			2nd and 3rd Instar	2nd Instar	3rd Instar
Diafil	10	5	52	84	83
Diafil	20	5	50	83	82
Diafil	30	5	43	82	81
Dicalite	10	5	50	84	82
Dicalite	20	5	50	78	82
Dicalite	30	5	50	82	81
Fine Perlite	10	5	58	85	84
Fine Perlite	20	5	55	84	83
Fine Perlite	30	5	48	84	81
Teufel Mix	---	5	50	71	65
Sunshine LC1 Mix	---	5	66	85	85
SB300 Universal	---	5	43	76	75
Mean (± SE)			51.2 ± 1.8	81.5 ± 1.2	80.3 ± 1.6
Range			43–66	71–85	75–85

Table 4. Mean adult fungus gnat (*Bradysia* sp. nr. *coprophila*) emergence based on yellow sticky card (2.5 × 2.5 cm) counts from growing medium samples initially inoculated with 2nd and 3rd instar fungus gnat larvae for growing media containing diatomaceous earth (DE) and other growing media tested for the second experiment. There were about 20 fungus gnat larvae used per replication.

Formulation name	n	Concn (lb DE/yard^3)	2nd Instar (mean ± SE)	3rd Instar (mean ± SE)
Diafil	7	10	7.5 ± 1.2 a[z]	4.3 ± 0.9 bc
Diafil	7	20	8.4 ± 2.1 a	4.1 ± 0.5 bc
Diafil	7	30	10.4 ± 1.7 a	3.8 ± 0.7 bc
Dicalite	7	10	8.7 ± 1.1 a	3.7 ± 0.9 bc
Dicalite	7	20	9.0 ± 1.4 a	2.0 ± 0.4 c
Dicalite	7	30	5.1 ± 0.8 a	3.3 ± 0.9 bc
Fine Perlite	7	10	5.7 ± 0.8 a	5.0 ± 1.5 b
Fine Perlite	7	20	7.2 ± 1.1 a	3.7 ± 0.8 bc
Fine Perlite	7	30	10.3 ± 1.4 a	3.3 ± 1.2 bc
Teufel Mix	7	---	6.1 ± 1.5 a	5.8 ± 1.0 b
Sunshine LC1 Mix	7	---	8.1 ± 1.6 a	3.7 ± 0.7 bc
SB300 Universal	7	---	9.0 ± 1.7 a	9.3 ± 1.1 a

[z]Means not followed by a common letter are significantly different (P = 0.05) as determined by Fisher's protected least significant difference (LSD) test.

the percent moisture content. Preliminary data have shown that excessive moisture levels may be harmful to fungus gnat larvae thus affecting adult emergence (Cloyd and Dickinson, unpublished data). Although not directly evaluated in this study, moisture content may also have influenced the efficacy of DE to control fungus gnats (Korunic, 1998) as DE has been shown to less effective under moist conditions (Maceljski and Korunic, 1971).

Based on the results from both experiments, the incorporation of DE into growing medium had no influence on the 2nd and 3rd instars of the fungus gnat, *B*. sp. nr. *coprophila*. This study has demonstrated that the use of DE as an amendment incorporated into growing media, at the concentrations tested, does not negatively affect fungus gnat larvae, which suggests that incorporating DE into growing medium may not be beneficial to greenhouse producers. However, further studies are needed to access whether there is differential larval susceptibility (first instar vs. later instars) to DE and if moisture content influences the efficacy of DE.

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