Effect of growing media and their constituents on fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) adults

RAYMOND A. CLOYD¹, AMY DICKINSON², RICHARD A. LARSON³ and KAREN A. MARLEY³

¹Department of Entomology, Kansas State University, Manhattan, Kansas, ²Division of Biodiversity and Ecological Entomology, Illinois Natural History Survey, Champaign, Illinois, and ³University of Illinois, Department of Natural Resources and Environmental Sciences, Urbana, Illinois, USA

> **Abstract** This study was conducted to determine the attractiveness of two growing media, commonly utilized in greenhouses, to fungus gnat, Bradysia sp. nr. coprophila adults. The constituents of the most attractive growing medium tested were determined by gas chromatography analysis using a steam-distillation procedure. We found that fungus gnat adults were more attracted to the growing medium, SB300 Universal Professional Growing Mix, which contains composted bark, than to another growing medium (Sunshine LC1 Mix) and their components when tested in a series of laboratory experiments using multiple-choice experimental arenas. A higher percentage of fungus gnat adults were attracted to moist SB300 (92%) than SB300 growing medium that had been oven dried (8%). In addition, fungus gnat adults preferred SB300 although they had been reared on Sunshine LC1 Mix. When comparing the SB300 fresh from the bag to growing medium that had been pasteurized and moistened with water, gas chromatographic-mass spectroscopic data showed there were declines in several terpenoid constituents as well as an increase in fatty acids and cyclosulfur. The results of this study indicate that B. sp. nr. coprophila adults prefer certain growing media, which may assist greenhouse producers in managing fungus gnats in crop production systems.

> **Key words** Attraction, fungus gnats, growing medium, pest management, preference, steam distillation DOI 10.1111/j.1744-7917.2007.00175.x

Introduction

Fungus gnats are major insect pests in greenhouses (Dennis, 1978, Hamlen & Mead 1979). Adults are primarily a nuisance causing minimal plant damage (Cloyd, 2000); however, the adult females lay eggs that hatch into larvae that are directly responsible for damaging plants by feed-ing on roots (Hungerford, 1916; Wilkinson & Daugherty, 1970; Fawzi & Kelly, 1982). Growing media vary in their

Correspondence: Raymond A. Cloyd, Department of Entomology, Kansas State University, 123 Waters Hall, Manhattan, KS 66506-4004, USA. Tel: 785 532 4750; fax: 785 532 6232; email: rcloyd@ksu.edu attractiveness to fungus gnat adults (Lindquist, 1994). However, studies have shown that fungus gnats are attracted to certain growing media. For example, Meers and Cloyd (2005) reported that fungus gnat, *Bradysia* sp. nr. *coprophila* Lintner (Diptera: Sciaridae) adults tended to lay eggs more often in Metro-Mix 560 (The Scott's Company; Marysville, OH, US) than either Sunshine LC1 Mix (Sungro Horticulture, Inc.; Bellevue, WA, US) or SB300 Universal Professional Growing Mix (Strong-Lite Horticulture Products; Pine Bluff, AK, US) growing media. Fungus gnat adults also appear to be more attracted to or prefer moist growing media containing peat moss (Baker, 1994) since moist growing medium may provide a higher level of fungal activity (Baker, 1994, Olson *et al.*, 2002), which is attractive to and serves as a food source for fungus gnat adults (Kennedy, 1974; Anas & Reeleder, 1988; Gardiner *et al.*, 1990).

Insect attractant and repellent chemicals of plant origin have been widely studied over the past 40 years (Edwards, 1980). However, minimal research has been conducted to determine the effects of such materials on fungus gnats, Bradysia spp. (Diptera: Sciaridae). Research on this genus by Xue et al. (2002) revealed that Bradysia odoriphaga Yang et Zhang (Diptera: Sciaridae) adults were attracted to garlic (Allium tuberosum Rottl. ex K. Spreng) and chive (Allium schoenoprasum L.) extracts. Ming et al. (2002) reported that B. odoriphaga adults were attracted to fresh chives; garlic (Allium sativum L.) in an alcohol extract, and calcium polysulfide. Related species in the family Chloropidae (also referred to as "eye gnats") are attracted to several types of naturally occurring chemicals including esters, long-chain alkyl ketones, indoles, tertiary amines, short chain fatty acids, and substituted phenols such as carvacrol (Snapp & Swingle, 1929; Hwang et al., 1976).

Research has suggested that inhibitory chemicals may be present in plant-based materials including components of growing media commonly used in greenhouses. For example, in a study evaluating 20 different growing medium combinations, it was reported that fewer *Bradysia* spp. adults emerged from growing media containing composted pine bark than the other growing media tested, including a hardwood bark-amended growing medium (Lindquist *et al.*, 1985). Pine essential oils, which contain an abundance of volatile terpenes, have been shown to repel insects and mites (Macchioni *et al.*, 2002).

In general, no studies have conducted extensive evaluations to determine if particular growing media and their volatile constituents are attractive to fungus gnat adults under laboratory conditions. As such, the purpose of this study was to determine the attractiveness of growing media and their constituents to the fungus gnat, *B*. sp. nr. *coprophila* adults. A series of laboratory experiments involving growing media, growing medium moisture content, alterations of growing medium, and differences in fungus gnat rearing procedures (based on growing medium used), were conducted to determine if fungus gnat adults are attracted to a particular growing medium used by greenhouse producers. In addition, a gas chromatography analysis was performed to identify characteristic volatile compounds in the most attractive growing medium.

Materials and methods

Two different growing media commonly used by greenhouse producers were tested: Sunshine SB300 Universal Professional Growing Mix and Sunshine LC1 Mix (Sun



Fig. 1 Six-armed experimental arena used for all experiments in the study. A 60×15 mm glass Petri dish containing the treatments (e.g. growing medium) was placed into the designated sample compartments (B). A 2.5×2.5 -cm yellow sticky card was placed onto the surface of each treatment. Adult fungus gnats, *Bradysia* sp. nr. *coprophila*, obtained from a laboratory colony, were collected in a plastic vial and then placed into the central compartment (A). The number of adult fungus gnats captured on the yellow sticky cards and on the floor in the sample compartments (B) was used to measure the attractiveness of each treatment.

Gro Horticulture, Inc.; Bellevue, WA, US). In addition, three growing medium components were evaluated: pea rock, sand, and perlite (Silbrico Comp.; Hodgkins, IL, USA). All experiments were conducted in the absence of a light source since fungus gnat adults are attracted to light (Cloyd *et al.*, 2007).

A set of five, six-armed experimental arenas were developed for this study (Fig. 1). Each experimental arena consisted of a central compartment made from a round, clear, 5.3 L polypropylene microwavable container (Flex & Seal® Rubbermaid, Inc., Fairlawn, OH, USA) 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. Each hole was fitted with a round, acrylic plastic, 2.8 cm long, hollow sleeve (internal diameter of 3.0 cm). These sleeves were permanently affixed to the central compartment using plastic cement. Hollow, clear acrylic tubes (internal diameter of 2.2 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 using the same sleeve as described above. These smaller compartments were clear, square, 1 L polycarbonate microwavable containers (Stain Shield® Rubbermaid, Inc., Fairlawn, OH, USA) with snap-on lids.

The experimental arenas were designed so that we could remove the sample compartments and cover the open sleeves with laboratory film (Parafilm[®] Pechiney Plastic Packaging; Menasha, WI, US) when conducting choice tests requiring less than six sample compartments. For example, when two-choice experiments were conducted, two sample compartments positioned directly across from each other were used and all the other sleeves were sealed off with laboratory film.

Each soilless growing medium and component treatment was placed into a 60×15 mm Pyrex glass Petri dish. The Petri dishes were filled to volume and then leveled off. A 2.5×2.5 cm yellow sticky card (Whitmire Micro-Gen; St. Louis, MO, US) was then placed on the surface of the growing medium or component to assess fungus gnat adult movement into the sample compartments. Treatments involving aqueous suspensions (12.0 mL) were also placed in a 60×15 mm Pyrex glass Petri dish, and a circular piece of thrips screening 50 \times 24 (0.2 \times 0.8 mm) (Greentek; Edgerton, WI, US) with a mesh size of 135μ m was glued across the opening of the Petri dish using a silicon-based glue-gun (High Temp Classic Jr., Adhesive Technologies, Inc.; Hampton, NH, US) in order to prevent the fungus gnat adults from drowning. The yellow sticky card was then placed on top of the screening.

Twenty-four hours before each experiment was initiated the individual growing media and composted pine bark treatments was placed into 708 mL plastic containers, filled to volume, and then leveled off. Approximately 200 mL of deionized water was then added, and the growing medium was thoroughly mixed by hand to obtain a homogenous mixture. The growing medium was then pasteurized for 5 minutes in a microwave oven (1200-W output) at full power in order to kill any larvae, pupae, or adult insects that may have initially contaminated the growing medium. Laboratory observations indicated that this time period for pasteurization was sufficient to kill any fungus gnat larvae and adults (Amy Dickinson, personal observation, 2006). None of the inert treatments (sand, perlite, pea rock) was pasteurized nor moistened.

Data on percent moisture content of the soilless growing media and pine bark treatments were collected and analyzed for each experiment. After the growing medium treatments were moistened, pasteurized, and placed into the 60×15 mm Pyrex glass Petri dishes, each sample was weighed before being placed in the experimental arena. At the completion of each experiment, the treatment samples were placed into an oven set at $60 \pm 1^{\circ}$ C. After approximately 7 days, the weight of the samples was constant and this final weight was recorded, and percent moisture content was calculated. The formula used to obtain percent moisture content was as follows: $(A-B) - (C-B)/(A-B) \times 100,$

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

Experimental procedures

Fungus gnat adults used in the study were obtained from laboratory colonies reared on either SB300 or LC1 Mix soilless growing media (Cabrera *et al.*, 2005). Fungus gnat adults used in all the experiments were 6–8 days old. Approximately 100 fungus gnat adults (mixture of females and males) were released into the central compartment of each experimental arena. Adults were aspirated into two 9dram vials containing approximately 50 adult fungus gnats each. Two vials were used instead of one in order to lessen possible injury and mortality due to crowding within a single vial. The vials were placed in the middle of the central compartment, the vial lids were removed, and then the central compartment lid was quickly sealed.

All experiments were conducted in an environmentally controlled walk-in chamber located in the National Soybean Research Center, Urbana, IL, US. The chamber was maintained at a temperature of $24 \pm 2^{\circ}$ C. The experimental arenas were placed on the floor of the chamber with the treatments arranged randomly within each experimental arena. Previous research had indicated that fungus gnat adults are attracted to light (Cloyd et al., 2007) so the lights within the chamber were turned off immediately after releasing the adults into the central component of each experimental arena. Fungus gnat adult distribution within the sample compartments was determined after 48 hours at which time the majority of fungus gnat adults were captured on the yellow sticky cards for each treatment (Dickinson, unpublished data, 2006). The number of adult fungus gnats per yellow sticky card per treatment was recorded. Fungus gnat adults that were on the floor of each sample compartment, and determined to be dead, were also recorded.

Steam distillation procedure

The constituents of the soilless growing medium were determined by gas chromatography (GC) analysis of the steam-distillation portion. Growing medium was placed into a tared 250 mL pear-shaped glass flask with gentle tamping to minimize air pockets, and then re-weighed to determine the actual amount. The top and bottom of the flask had extended 24/40 standard taper joints to connect to the boiling apparatus at the bottom and the condenser at the top. Nylon netting was placed in the bottom of the flask to prevent debris from falling into the boiling container. Steam was generated by boiling water, which was passed through the growing medium and then condensed by a water-cooled glass condenser into a receiving flask. A typical procedure lasted approximately 1 hour and 200 mL of aqueous steam distillate (ASD) was collected.

Using a glass separatory funnel, the ASD (100 mL) was extracted twice into 30-mL portions of methylene chloride (liquid-liquid extraction). To enhance detection by GC, the methylene chloride extract was reduced in volume, using a Kuderna-Danish apparatus, which gently evaporates the solvent to a minimum volume of 1.0 mL (Environmental Protection Agency, 1996). In order to improve detection of polar constituents such as dicarboxylic and fatty acids, samples were methylated using 6 mol/L HCl in methanol (Patnaik & Khoury, 2005), treated to reduce the solvent, and redissolved in methylene chloride.

A Hewlett Packard 5890 Series II gas chromatograph with a flame ionization detector (FID) was used for quantitative analysis. Two microliter injections were made onto a DB-5 capillary column (0.32 mm inside diameter \times 30 m) (J & W Scientific Co; Rancho Cordova, CA, US). Output was recorded on a ChromJet 4400 integrator (Spectra-Physics; San Jose, CA, US).

For compound identification, an Agilent 6890N GC with a 5973N series mass selective detector (MSD) was used (Agilent Technologies; Palo Alto, CA, US). One microliter injections were made onto a HP-5 capillary column (0.25 mm inside diameter \times 30 m) (Agilent Technologies; Palo Alto, CA, US). National Institute for Standards and Technologies searching algorithms and manual interpretation provided reasonable matching to the obtained mass spectroscopic fragmentation patterns. Authentic standards of commercially available compounds were obtained from Sigma-Aldrich (St. Louis, MO, US) and matched with GC retention times (FID and MSD) and mass spectra for confirmation.

High-purity organic solvents (reagents and chemicals) were purchased from Fisher Scientific (Fairlawn, NJ, US). High-purity water was prepared from in-house reverse osmosis treated water, which was then glass distilled from an alkaline solution of potassium permanganate.

Experiment 1: Attraction of fungus gnat, Bradysia sp. nr. coprophila *adults to growing media*

This experiment, using the five six-armed experimental arenas, consisting of six treatments with 10 replications per treatment (growing media or components) for each choice test, assessed the attractiveness of two different growing media and components to fungus gnat adults. The experimental procedures were as described above. The six treatments were: SB300, LC1 Mix, perlite, sand, pea rock and an empty Petri dish covered with a lid.

Experiment 2: Effect of growing medium moisture content in attracting fungus gnat, Bradysia sp. nr. coprophila adults

This experiment, consisting of two treatments with 10 replications per treatment for each choice test, evaluated the effect of growing medium moisture content in attracting fungus gnat adults based on a two-choice assessment. Both treatments included SB300 growing medium. One treatment involved using the growing medium directly from the bag and unaltered (without pasteurization or adding water). The second treatment was unaltered as well; however, the growing medium was dried for 7 days in an oven at $60 \pm 1^{\circ}$ C until all the moisture in the growing medium had evaporated.

Experiment 3: Attraction of SB300 growing medium and composted pine bark to fungus gnat, Bradysia sp. nr. coprophila adults

This experiment consisted of two treatments with 10 replications per treatment for each choice test, and was designed to determine the attractiveness of SB300 and composted pine bark, which is a component of SB300. A bag of composted pine bark, which comprises 50% of SB300, was supplied directly from the manufacturer, Sun Gro Horticulture Company (Bellevue, WA, US).

Experiment 4: Fungus gnat, Bradysia sp. nr. coprophila adult attraction to standard SB300 and SB300 growing medium subject to a steam distillation process

This experiment consisted of two treatments with 10 replications per treatment for each choice test. The experiment tested the effect of SB300 subject to a steam distillation process (described previously) intended to remove volatiles, and then comparing the growing media's attraction to fungus gnat adults with SB300 that had not undergone the steam distillation process.

Experiment 5: Attraction of fungus gnat, Bradysia sp. nr. coprophila adults to two growing media (SB300 and LC1 Mix) when reared to adults on LC1 Mix

This experiment involved two treatments with 10 replications per treatment for each choice test, and was designed to test the attractiveness of the growing media SB300 and LC1 Mix when fungus gnats were reared to adults on LC1 Mix in order to assess if conditioning was involved in attraction. Approximately 100 fungus gnat adults, which were 6-9 days old (mixture of females and males), were released into the central compartment of each experimental arena for this two-choice experiment.

Data analysis

Data was calculated per replicate as a percent of the number of fungus gnat adults captured on the yellow sticky cards as well as those on the floor of each sample compartment using a statistical software program (SAS Systems for Windows, version 8.2). For experiment one, data were normalized by arcsine square-root transformation and subjected to a one-way analysis of variance (ANOVA) with sample compartment as the main effect (SAS Institute, 2002). Significant sample compartment means were separated using a Fisher's protected least significant difference (LSD) test at P = 0.05. For experiments two through five, data were normalized by arcsine square-root transformation and a t-test procedure (SAS Institute, 2002) was conducted to determine significant differences between the two treatments. All data presented are non-transformed.

Results

Experiment 1: Attraction of fungus gnat, Bradysia sp. nr. coprophila *adults to growing media*

Treatment was significant (F=133.33; df = 5,59; P < 0.000 1) with a significantly higher percentage of fungus gnat adults present in the sample compartments containing SB300 and LC1 Mix growing media than the other treatments (Table 1). In addition, the SB300 growing medium was significantly different from the LC1 Mix with nearly twice as many fungus gnat adults, based on percent, present in the sample compartment containing SB300 (Table 1).

The mean percent moisture content of the SB300 and LC1 Mix were 61% and 74%, respectively (Table 1).

Experiment 2: Effect of growing medium moisture content in attracting fungus gnat, Bradysia sp. nr. coprophila adults

Treatment was significant (F = 6.23; df = 9,18; P < 0.000 1) with a higher percentage of fungus gnat adults present in the sample compartments containing unaltered SB300 (92%) compared to those containing oven dried SB300 (8%). The mean percent moisture content of the unaltered SB300 was 54%; whereas, the mean percent moisture content of the oven-dried SB300 was 1%.

Experiment 3: Attraction of SB300 and composted pine bark to fungus gnat, Bradysia sp. nr. coprophila adults

There was a significant effect of treatment (F = 2.06; df = 9,18; P < 0.000 1) with a higher percentage of fungus gnat adults present in the sample compartments with SB300 (70%) than those containing composted pine bark (30%). The mean percent moisture content of the SB300 and the dry composted pine bark was 61% and 65%, respectively.

Experiment 4: Fungus gnat, Bradysia sp. nr. coprophila adult attraction to standard SB300 and SB300 growing medium subject to a steam distillation process

Treatment was not significant (F = 1.06; df = 9,18; P = 0.689) as there was no difference in the percentage of fungus gnat adults recovered in either the sample compartments with SB300 (51%) or steam distilled SB300 (49%). The mean percent moisture content for the SB300 that had undergone the steam distillation process was 75%, and the mean percent moisture content for the SB300 not steam distilled was 65%.

Treatment	n	MC^\dagger	Mean (\pm SEM) percent fungus gnat adults recovered
SB300 Universal Mix	10	61%	$50 \pm 3a^{\ddagger}$
Sunshine LC1 Mix	10	74%	$28 \pm 2b$
Rock	10	0%	$4 \pm 1c$
Sand	10	0%	$5 \pm 1c$
Perlite	10	0%	$7 \pm 1c$
Empty Petri dish	10	_	$6 \pm 0c$

Table 1 Mean (\pm SEM) percent fungus gnat, *Bradysia* sp. nr. *coprophila* adults recovered in the sample compartments of each experimental arena for each treatment in experiment one, and percent moisture content of the soilless growing media tested.

 $^{\dagger}MC = Percent moisture content.$

^{*}Means followed by common letter are not significantly different based on a Fisher's protected least significant difference (LSD) test with P < 0.05.

Experiment 5: Attraction of fungus gnat, Bradysia sp. nr. coprophila adults to two growing media (SB300 and LC1 Mix) when reared to adults on LC1 Mix

There was a significant effect of treatment (F = 1.03; df = 9, 18; P < 0.000 1) with a higher percentage of fungus gnat adults recovered in the sample compartments containing SB300 (61%) compared to the sample compartments with LC1 Mix (39%). The mean percent moisture content of the growing media SB300 and LC1 Mix was 68% and 74%, respectively.

Components of growing media

Typical of flavor and odor analyses, the resulting chromatograms revealed a complex mixture, with at least 20– 40 constituents, for the SB300 growing medium and the main components. A generalized summary is presented in Table 2, which provides an outline of the types of compounds currently identified and an approximation of their relative amounts. While the growing medium and components can all be characterized as having a musty odor, there

Table 2 Percent composition of volatiles detected in SB300 Universal Professional Growing Mix and two components using a steam distillation procedure as determined by gas chromatography (GC) analysis.

Compound	Composted bark	Peat moss	SB300 Universal Mix	SB300 Universal Mix
	(Dry)	(Dry)	(Dry)	(AP)
Furfural	15.7	9.60	4.70	NP
5-methylfurfural	5.1	3.60	13.10	NP
Dicarboxylic acids $(C_4 - C_9)^{\dagger}$	NP	Minor	NP	NP
Alkanoic fatty acids [†]				
C ₆	NP	1.40	NP	NP
C ₇	NP	0.70	NP	NP
C ₈	1.2	2.30	< PT	NP
	1.0	1.20	1.10	NP
C ₁₀	< PT	0.57	< PT	<pt< td=""></pt<>
C ₁₁	NP	< PT	< PT	<pt< td=""></pt<>
C ₁₂	NP	2.30	< PT	NP
C ₁₄	NP	0.86	< PT	0.95
C ₁₆	3.9	1.80	NP	3.70
C ₁₈	NP	0.75	NP	12
C ₁₆ ene fatty acid	NP	NP	NP	Minor
Cyclosulfur (S_8)	NP	0.96	< PT	20.7
Camphor	< PT	NP	3.10	0.75
Borneol	2.6	NP	0.63	3.60
Alpha-terpineol	1.5	NP	13.6	< PT
Verbenone	NP	NP	20.5	< PT
Sesquiterpenes	Minor	NP	Minor	NP
Alkyl alcohols [†]	Minor	NP	Minor	Minor
1,3-dimethoxybenzene	NP	6.90	NP	NP
Acetophenone	NP	NP	2.60	1.20
Benzaldehyde	< PT	1.20	3.60	6.30
Dodecanol	NP	1.10	2.80	NP
Abietic acid	Minor	NP	NP	NP
Labdadienes	Minor	NP	NP	NP
Total number compounds detected	ed > 40	> 60	~30	25

AP = As prepared: microwaved and moistened.

[†]Determined as methyl ester.

< PT = below peak threshold based on gas chromatography-flame ionization detector, but confirmed by gas chromatography-mass selective detector.

NP = Not present based on gas chromatography-flame ionization detector or gas chromatography-mass selective detector.

are distinct differences that can be determined using a steam-distilled fraction. For example, peat moss contains a series of dicarboxylic acids (C4–C8), such as succinic acid and suberic acid, but not the terpene-derived compounds, which are characteristic of pine bark. The condition of the growing medium or components such as the degree of moistness or age also resulted in changes in the volatile composition. When comparing SB300 fresh from the bag versus growing medium that had been prepared for the experiments (pasteurized and moistened with water), there was a reduction in some of the terpenoid constituents as well as an increase in several of the fatty acids and cyclosulfur (Table 2); however, there was no effect on attractiveness to fungus gnat adults.

Discussion

This study has demonstrated that fungus gnat adults are highly attracted to the SB300 growing medium, more so than Sunshine LC1 Mix, based on the results from the first experiment, which is the reason why in subsequent experiments we only used SB300 growing medium to evaluate attractiveness. In addition, even when fungus gnats were reared on LC1 Mix growing medium, adults were still attracted to SB300 (experiment 5) indicating that there was no effect of conditioning, which may have confounded the results since conditioning during the rearing process may alter the response of insects (Boller, 2006).

Fungus gnat adults, in general, are attracted to moist growing media, and are less attracted to growing media with low moisture contents (< 10%). However, moisture content failed to influence the fungus gnat adults in most of the experiments conducted in our study. For example, although the LC1 Mix had a higher moisture content (74%) than the SB300 (61%), significantly more fungus gnat adults were present in those sample compartments with SB300. The SB300 growing medium contains composted bark (50%), Canadian sphagnum peat moss (20%), perlite (10%), medium coarse vermiculite (20%), Dolomitic lime, gypsum, and wetting agents, whereas the LC1 Mix contains Canadian sphagnum (75%), perlite (25%), Dolomitic lime, gypsum, and wetting agents. The major difference between these two growing media is the absence of composted bark in the LC1 Mix. In our study, we found fungus gnat adults to be highly attracted to SB300, which may be due to the presence of the composted bark. In contrast, Lindquist et al. (1985) reported that fungus gnats were less abundant in growing media containing composted pine bark than the other growing media evaluated, which included a hardwood bark amended growing medium. However, this study was conducted in a greenhouse environment, which is less stringent than laboratory conditions, and relied on natural fungus gnat infestations.

The use of an experimental arena, when conducting laboratory experiments, such as the one utilized in our study, is a common method to evaluate attraction of compounds to insects (Gothilf & Galun, 1982; Martin et al., 1990). Furthermore, Choudhury (1982) indicated the importance of conducting experiments in the laboratory to assess insect responses, which typically eliminates any unknown variables or confounding factors that may occur under field conditions. In addition, we used types of plastics (polypropylene and acrylic) that do not emit volatiles. The reason why we pasteurized the growing media prior to use in the experiments is that fungus gnats have been shown to be present in bagged soilless growing media (Cloyd & Zaborski, 2004). The pasteurization process killed the larvae and pupae, which avoided the possibility of confounding our results. However, it is not known if this process changed any properties of the growing medium although data from the gas chromatograph indicated there were a decline in several terpenoid constituents and an increase in fatty acids and cyclosulfur.

Steam distillation was chosen as the method to extract the constituents of the soilless growing medium in order to produce material that could be used directly in testing with the fungus gnats, as well as allow for further purification and identification by gas chromatography (GC) analysis. Gas chromatography analysis results (not presented) consisted of complex chromatograms with a diverse variety of compounds; however, very few predominant components were evident.

The major components of the soilless growing medium were plant-based products. It is well documented that selective extractions of plant material using a variety of techniques will always yield an array of soluble compounds that are detectable with modern chromatographic methods. In general, characteristic groups of compounds may be associated with a particular plant, such as phenolics or terpenes. Cellular breakdown products such as acids, aldehydes, ketones, and alcohols may also be detected in these extracts, although at much lower quantities. Extracts of peat moss and composted pine bark, which are considered to be already partially decomposed, may yield numerous small molecular weight compounds indicative of cellular disruption or degradation.

Fatty acids, usually the smaller (C1–C5), more pungent types that are typically found in odoriferous composted material were not detected in the growing medium; instead, C6–C18 unsaturated fatty acids were present. Dicarboxylic acids (C4–C9) were present in the peat moss material. Expected constituents of pine bark, including the monoterpenes, camphene and pinene were not detected; however, terpene alcohols, such as borneol and alphaterpineol, which are considered to be microbial transformation products of camphene and limonene, respectively (Braddock & Cadwallader, 1995), were present in abundant quantities (Table 2).

Peat moss, which is a universal amendment in many soilless growing media, is a partially decomposed material that accumulates under the living layers of sphagnum moss. However, no odor or volatile studies have been performed using this material. The soluble components of sphagnum moss have been identified as phenolics (Verhoeven & Liefveld, 1997), which is likely the result of the breakdown of condensed tannins. Two volatile aromatic compounds were discovered in the soilless growing medium extracts, acetophenone and benzaldehyde (Table 2), both of which have been detected in composted material (Poehle & Kliche, 1996; Tolvanen et al., 1998). Another marker associated with microbial transformation is indicated by the presence of S_o (cyclooctasulfur) in peat moss (Table 2), which is likely formed during the oxidation of bacteria on hydrogen sulfide, a well-known odoriferous component of degraded waste materials (Steudel, 1996).

This study was designed to determine the attractiveness of growing media to fungus gnat adults, and we have demonstrated that Bradysia sp. nr. coprophila adults do prefer Sunshine SB300 Universal Professional Growing Mix over the Sunshine LC1 Mix. However, further research is needed to evaluate other growing media and the volatiles emitted by composted bark in order to assess which constituents are most attractive to fungus gnat adults. Differences in attraction may be due to variability in volatile growing medium constituents as shown in Table 2, and future work will focus on evaluating specific compounds or mixtures characteristic of the growing medium. If the most attractive constituents can be identified, then this may lead to the development of an attractant that can be used to lure fungus gnats, which will assist greenhouse producers in managing fungus gnats in greenhouse production systems.

Acknowledgments

We wish to acknowledge the Fred C. Gloeckner Foundation for providing financial support for this study.

References

- Anas, O. and Reeleder, R.D. (1988) Feeding habits of *Bradysia coprophila* on fungi and plant tissue. *Phytoprotection*, 69, 73–78.
- Baker, J.R. (1994) Insects and related pest of flowers and foliage

plants: Some important, common and potential pests in the southeastern United States. Ag-136. North Carolina Cooperative Extension Service, 4 pp.

- Boller, E. (2006) Behavioral aspects of mass-rearing of insects. *BioControl*, 17, 9–25.
- Braddock, R.J. and Cadwallader, K.R. (1995) Bioconversion of citrus d-limonene. *Fruit Flavors: Biogenesis, Characterization and Athentication* (eds. R.C. Rouseff & M.M. Leahy), pp. 142–147. ACS Symposium Series 596. American Chemical Society, Washington, D.C.
- Cabrera, A.R., Cloyd, R.A. and Zaborski, E.R. (2005) Development and reproduction of *Stratiolaelaps scimitus* (Acari: Laelapidae) with fungus gnat larvae (Diptera: Sciaridae), potworms (Oligochaeta: Enchytraeidae) or *Sancassania* aff. *sphaerogaster* (Acari: Acaridae) as the sole food source. *Experimental Applied Acarology*, 36, 71–81.
- Choudhury, J.H. (1982) Design of a horizontal olfactometer to test the responses of small insects during flight. *Proceedings of the 3rd National Zoological Conference*, pp. 82–86. March 15–17, 1981. Dacca, Bangladesh.
- Cloyd, R.A., Dickinson, A., Larson, R.A. and Marley, K.A. (2007) Phototaxis of fungus gnat, *Bradysia* sp. nr. *coprophila* (Lintner) (Diptera: Sciaridae), adults to different light intensities. *HortScience*, 42, 1217–1220.
- Cloyd, R.A. and Zaborski, E.R. (2004) Fungus gnats, *Bradysia* spp. (Diptera: Sciaridae), and other arthropods in commercial bagged soilless growing media and rooted plant plugs. *Journal* of Economic Entomology, 97, 503–510.
- Cloyd, R.A. (2000) Fungus gnat and shorefly management strategies: panel discussion. *Proceedings of 16th Conference* on Insect and Disease Management on Ornamentals (eds. A. I. King & I.D. Greene), pp. 57–59. Society of American Florists, Alexandria, VA.
- Dennis, D.J. (1978) Observations of fungus gnat damage to glasshouse cucurbits. *New Zealand Journal of Experimental Agriculture*, 6, 83–84.
- Edwards, P.J. (1980) *Ecology of Insect-Plant Interactions*. Arnold, London.
- Environmental Protection Ageny (1996) *Method 3510C, in SW-*846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, 3rd edition, U.S. EPA, Washington, D.C.
- Fawzi, T.H. and Kelly, W.C. (1982) Cavity spot of carrots caused by feeding of fungus gnat larvae. *Journal of American Society of Horticultural Science*, 107, 1177–1181.
- Gardiner, R.B., Jarvis, W.R. and Shipp, J.L. (1990) Ingestion of *Pythium* spp. by larvae of the fungus gnat *Bradysia impatiens* (Diptera: Sciaridae). *Annuals of Applied Biology*, 116, 205–212.
- Gothilf, S. and Galun, R. (1982) Olfactometer and trap for evaluating attractants for the Mediterranean fruit fly, *Ceratitis capitata. Phytoparasitica*, 10, 79–84.
- Hamlen, R.A. and Mead, F.W. (1979) Fungus gnat larval control

in greenhouse plant production. *Journal of Economic Entomology*, 72, 269–271.

- Hungerford, H.B. (1916) Sciari maggots injurious to potted plants. *Journal of Economic Entomology*, 9, 538–549.
- Hwang, Y.S., Mulla, M.S. and Axelrod, H. (1976) Effects of structural modifications on the activity of *Hippelates* eye gnat attractants and coattractants. *Environmental Entomology*, 5, 65–67.
- Kennedy, M.K. (1974) Survival and development of *Bradysia impatiens* (Diptera: Sciaridae) on fungal and non-fungal food sources. Annals of the Entomological Society of America, 67, 745–749.
- Lindquist, R.K. (1994) Integrated management of fungus gnats and shore flies. Proceedings for the 10th Conference of Insect and Disease Management on Ornamentals (ed. K. Robb), pp. 58–67. Society of American Florists. Alexandria, VA.
- Lindquist, R.K., Faber, W.R. and Casey, M.L. (1985) Effect of various soilless root media and insecticides on fungus gnats. *HortScience*, 20, 358-360.
- Macchioni, F., Cioni, P.L., Falmini, G., Morelli, I. and Perrucci, S. (2002) Acaricidal activity of pine essential oils and their main components against *Tyrophagus* putrescentiae, a stored food mite. *Journal of Agricultural and Food Chemistry*, 50, 4586–4588.
- Martin, Jr., W.R., Nordlund, D.A. and Nettles, Jr., W.C. (1990) Response of parasitoid *Eucelatoria bryani* to selected plant material in an olfactometer. *Journal of Chemical Ecology*, 16, 499–508.
- Meers, T.L. and Cloyd, R.A. (2005) Egg-laying preference of female fungus gnat *Bradysia* sp. nr. *coprophila* (Diptera: Sciaridae) on three different soilless substrates. *Journal of Economic Entomology*, 98, 1937–1942.
- Olson, D.L., Oetting, R.D. and van Iersel, M.W. (2002) Effect of soilless potting media and water management on development

of fungus gnats (Diptera: Sciaridae) and plant growth. *HortScience*, 37, 919–923.

- Patnaik, P. and Khoury, J.N. (2005) Determination of acid herbicides in water by GC-MS: a modified method using single extraction and methanol esterification. *American Laboratory*, 12, 14–15.
- Poehle, H. and Kliche, R. (1996) Emission of odor substances from composting processes. *Zentralblatt für Hygiene und Umwelmedizin*, 199, 38–50.
- SAS Institute (2002) SAS/STAT Users' Guide, v. 8.2. SAS Institute, Inc., Cary, NC.
- Snapp, O.I., and Swingle, H.S. (1929) Preliminary report on attractants for peach insects. *Journal of Economic Entomology*, 22, 98–101.
- Steudel, R. (1996) Mechanism for the formation of elemental sulfur from aqueous sulfide in chemical and microbiological desulfurization processes. *Industrial and Engineering Chemistry Research*, 35, 1417–1423.
- Tolvanen, O.K., Hanninen, K.I., Veijanen, A. and Villberg, K. (1998) Occupational hygiene in biowaste composting. Waste Management and Research, 16, 525–540.
- Verhoeven, J.T.A. and Liefveld, W.M. (1997) The ecological significance of organochemical compounds in *Sphagnum*. *Acta Botanica Neerlandica*, 46, 117–130.
- Wilkinson, J.D. and Daugherty, D.M. (1970) The biology and immature stages of *Bradysia impatiens* (Diptera: Sciaridae). *Annals of Entomological Society of America*, 63, 656–660.
- Xue, M., Yuan, L. and Xu, M.L. (2002) The olfactory response of adults to volatiles and compare of toxicity of different insecticides to the adults and larvae of *Bradysia odoriphaga*. *Chinese Journal of Pesticide Science*, 4(2), 50–56. (in Chinese)

Accepted June 11, 2007