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Bounce® Fabric Softener Dryer Sheets Repel Fungus Gnat, *Bradysia* sp. nr. *coprophila* (Diptera: Sciaridae), Adults

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Abstract. **This study was conducted to assess the repellency of Bounce® original brand fabric softener dryer sheets against fungus gnat, *Bradysia* sp. nr. *coprophila* (Diptera: Sciaridae), adults. For all five experiments conducted under laboratory conditions, fungus gnat adults collected in the sample compartments that included Bounce® original brand fabric softener dryer sheets ranged between 12% and 18% compared with the mean proportion of fungus gnat adults recovered from sample compartments that excluded dryer sheets, ranging in mean proportion from 33% to 48%. Chemical analysis using a steam distillation procedure to isolate volatile constituents found linalool as one of the major volatiles detected in the Bounce® original brand fabric softener dryer sheets. Additional constituents isolated were benzyl acetate, beta-citronellol, and hedione. Based on the results from our study, under laboratory conditions, Bounce® fabric softener dryer sheets do in fact repel *B*. sp. nr. *coprophila* adults.**

Fungus gnats (*Bradysia* spp.) are common insect pests of greenhouse-grown crops (Dennis, 1978; Hamlen and Mead, 1979). The adult flies are considered a nuisance causing minimal direct plant damage (Cloyd, 2000); however, eggs laid by adult females hatch into larvae that directly injure plants by feeding on the roots (Fawzi and Kelly, 1982; Hungerford, 1916; Jarvis et al., 1993; Kennedy, 1971; Wilkinson and Daugherty, 1970). The primary means of dealing with fungus gnat populations in greenhouse production systems includes cultural management such as eliminating algae and avoiding overwatering (Ellisor, 1934; Keates et al., 1989), using insecticides (Cloyd and Dickinson, 2006; Hamlen and Mead, 1979; van Epenhuijsen et al., 2001), and/or biological control by introducing biological control agents (Birken and Cloyd, 2007; Chambers et al., 1993; Gillespie and Quiring, 1990; Harris et al., 1995). Another potential management strategy may be to use products or compounds with repellent activity, which could prevent adult females from laying eggs in growing media, thus reducing fungus gnat larval populations.

According to Dethier (1947), repellency is associated with any stimulus that elicits an avoidance reaction. Repellent products or compounds have been evaluated against insect pests, in particular, different species of biting mosquitoes (Fradin and Day, 2002; Granett, 1940; Rutledge et al., 1983). In addition, there are a number of plant-derived or similar compounds that have been tested to determine their repellent activity against mosquitoes (Barnard, 1999; Moore et al., 2007; Sharma et al., 1993; Trigg, 1996; Tunon et al., 1994). For years, master gardeners have claimed that placing Bounce® original brand fabric softener dryer sheets into the pockets of clothing repels mosquitoes (RAC, personal observation). Bounce® original brand fabric softener dryer sheets (Procter and Gamble, Cincinnati, OH), which contain biodegradable cationic softeners and perfume, are added to dryers to control static cling and give clothes a fresh scent (http://www.bouncesheets.com/en.us/products/original/detail.jsp?section=scents&scent=outdoorfresh).

In fact, Bounce® original brand fabric softener dryer sheets have been promoted to repel mosquitoes and "gnats" in trade magazines (Trumble, 2002); however, there are no quantitative data to substantiate these claims. As such, the objectives of this study were to 1) determine, under laboratory conditions, if Bounce® original brand fabric softener dryer sheets repel fungus gnat, *Bradysia* sp. nr. *coprophila* adults; and 2) determine the volatile constituents in the dryer sheets.

Materials and Methods

Bounce® original brand fabric softener dryer sheets and adult fungus gnat (Bradysia sp. nr. coprophila) experimental procedure. A series of five replicated experiments were conducted to ascertain whether Bounce® original brand fabric softener dryer sheets (Outdoor Fresh Scent™; Procter and Gamble) repel fungus gnat adults. All experiments were conducted in darkness because fungus gnat adults are attracted to light (Cloyd et al., 2007a). The equipment and methodology used in the experiments were similar to those described by Cloyd et al. (2007b), which involved the use of a set of five, six-armed experimental arenas consisting of a central compartment made from clear, round 5.3-L polypropylene microwavable container (Flex & Seal®; Rubbermaid, Inc., Fairlawn, OH) and six smaller compartments referred to as sample compartments, which were attached to the central compartment by plastic adjustable sleeves. Sample compartments were clear, square, 1-L polycarbonate microwavable containers (Stain Shield®; Rubbermaid, Inc.) accompanied with snap-on lids (Fig. 1). In the two-choice experiments, two sample compartments, positioned directly across from each other, were used, and the sleeves associated with the remaining four sample compartments were sealed off with laboratory film.

All experiments were conducted in a laboratory room at Kansas State University (Manhattan, KS). Each of the five experiments was blocked over time (2 d) to obtain 10 replications per treatment with five experimental arenas used per day or block. The room temperature was 24 ± 3 °C. The experimental arenas were placed on a table (1.8 × 0.6 m). A 760-mL round plastic container (Rubbermaid, Inc.) was filled with SB300 Universal Professional Growing Mix growing medium (Strong-Lite Horticultural Products, Pine Bluff, AR). Approximately 200 mL of deionized water was added to the growing medium, and then the growing medium was thoroughly mixed. The container with the growing medium was heated in a microwave oven at full power (850-W output) for 10 min. After heating, the container was removed and then the growing medium was allowed to cool for 15 min. Twenty milliliters of the growing medium was placed into a glass petri dish (5.0 cm in diameter). There were a total of 10 petri dishes used for each experiment (described subsequently). A small piece (5.0 × 5.0 cm) of the Bounce® original brand fabric softener dryer sheets ("dryer sheets") was used in the experiments. The growing medium, SB300 Universal Professional Growing Mix, was used in the designated experiments. Latex examining gloves (Fisher Scientific, Pittsburgh, PA) were worn when handling the dryer sheets to

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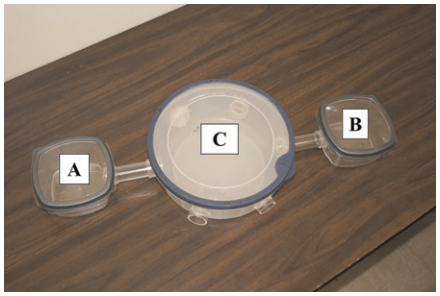

Fig. 1. Six-armed experimental arena used for all experiments in the study, in which just two arms were used for the two-choice experiments associated with the study. A glass petri dish (5.0 cm in diameter) containing the treatments was placed into the designated sample compartments (**A** or **B**). Adult fungus gnats, *Bradysia* sp. nr. *coprophila*, obtained from a laboratory colony, were collected in a 9-dram plastic vial and then released into the central compartment (**C**). The number of adult fungus gnats captured on 2.5 × 2.5-cm yellow sticky cards associated with each treatment was counted, and fungus gnat adults on the floor in the sample compartments (**A** or **B**) were counted. Also, the number of fungus gnat adults that remained in the central compartment (**C**) was recorded.

ensure that no human body odors contaminated the dryer sheets and thus influenced the results. The dryer sheets were positioned on the surface of the growing medium, and 20 mL of water was applied to the petri dishes associated with Expts. 1 and 5. We purchased new dryer sheets, which were used within 2 d of conducting each experiment.

The following experiments were performed in the study: Expt. 1 (dryer sheet versus water); Expt. 2 (dryer sheet versus moist growing medium); Expt. 3 (dryer sheet and moist growing medium versus moist growing medium); Expt. 4 (dryer sheet versus moist growing medium); and Expt. 5 (dryer sheet and growing medium versus dryer sheet and water). Expts. 2 and 4 were repetitions of the same experiment so as to ensure homogeneity of the data set obtained.

Each petri dish was covered with a metal lid fastened with antivirus insect screening 50 × 24 [0.2 × 0.8 mm (0.008 × 0.031 inch); Greentek, Edgerton, WI] that was secured to the inside of the rim with a rubber O-ring. A 2.5 × 2.5-cm yellow sticky card (Hummert International, Topeka, KS) square was glued onto the inside portion of the insect screening. Then each covered petri dish was placed inside the sample compartment.

Fungus gnat adults used in the study were obtained from laboratory colonies reared on SB300 Universal Professional Growing Mix similar to Cabrera et al. (2005). Specimens from the colony were identified as *Bradysia* sp. nr. *coprophila* (Lintner) by Raymond J. Gagne, Systematic Entomology Department Laboratory, U.S. Department of Agriculture. Voucher specimens are located in the Illinois Natural History Survey Insect Collection (#22896-22932, 32015-32021). Adult fungus gnats used in all the experiments were 6 to 9 d old. Approximately 150 fungus gnat adults (mixture of females and males; sex ratio unknown) were released into the central compartment of each experimental arena. Adults were aspirated into a 9-dram plastic vial (Bio-Quip Products, Rancho Dominguez, CA). The vial was placed in the middle of the central compartment, the vial lid was removed, and then the central compartment lid was quickly sealed.

Previous research has shown that fungus gnat adults are attracted to light (Cloyd et al., 2007a) so the vials containing adult fungus gnats were placed into the central compartments with the room door slightly ajar (so it was possible to initiate the procedure of releasing the fungus gnat adults into the central compartment); after all the vials had been placed into the central compartments, the individual left the room and the door was closed.

Fungus gnat adult distribution within the sample compartments was determined after 48 h. 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. Any adult fungus gnats flying around within the compartment were collected with the use of an aspirator and then the number was recorded. The number of adult fungus gnats, either live or dead, in the central compartment was also recorded.

Data analysis. Data were calculated per replicate as a mean proportion associated with the number of fungus gnat adults captured on the yellow sticky cards as well as those on the floor and flying around in each sample compartment of the total number collected from the sample compartments using a Statistical Software Program (SAS Systems for Windows, Version 9.1; SAS Institute Inc., Cary, NC). Data were normalized by arcsine square-root transformation and treatment means were compared by a Satterthwaite *t*-test procedure for unequal variance (SAS Institute 2002). Data were considered significant when the *P* value was ≤ 0.05. All data presented are non-transformed.

Steam distillation extraction experimental procedure. The volatile constituents of the dryer sheets were determined by gas chromatography analysis of the steam-distillation portion, similar to previous research (Cloyd et al., 2007b). The dryer sheets were folded and placed in a pear-shaped glass flask. Steam was generated and allowed to pass through the flask and condensed by a water-cooled glass condenser into a receiving flask. The aqueous condensate was extracted with methylene chloride, from which a 1-µL portion was used for the analysis by gas chromatography.

For compound identification, a Hewlett Packard 5890 gas chromatograph (Palo Alto, CA) connected to a 70-VSE Micromass spectrometer was used with split-less injections onto an Agilent J & W DB-5 capillary column (0.25 mm i.d. × 30 m) (Agilent Technologies, Santa Clara, CA). 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) and matched with gas chromatograph retention times and mass spectrometry for confirmation.

Results

Bounce® original brand fabric softener dryer sheets and adult fungus gnat (*Bradysia* sp. nr. *coprophila*) experiments

Expt. 1: Dryer sheet versus water. The range of fungus gnat adults collected from the colonies and used in the experiment was between 112 and 217. Treatment was significant ($t = 3.93$; df = 9, 17.7; $P = 0.0010$) with a higher proportion of fungus gnat adults present in the sample compartments containing water (42%) compared with those with the dryer sheets (16%).

Expt. 2: Dryer sheet versus moist growing medium—SB300 Universal Professional Growing Mix. The range of fungus gnat adults collected from the colonies and used in the experiment was between 120 and 239. Treatment was significant ($t = 7.70$; df = 9, 16.4; $P \leq 0.001$) with a higher proportion of fungus gnat adults present in the sample compartments containing the moist growing medium (48%) compared with the dryer sheet (12%).

Expt. 3: Dryer sheet and moist growing medium versus moist growing medium. The range of fungus gnat adults collected from the colonies and used in the experiment was between 138 and 220. Treatment was significant ($t = 6.09$; df = 9, 15.2; $P \leq 0.001$) with a higher proportion of fungus gnat adults present in the sample compartments containing only moist growing medium (45%) compared with sample compartments with a dryer sheet and moist growing medium (18%).

Expt. 4: Dryer sheet versus moist growing medium. The range of fungus gnat adults collected from the colonies and used in the experiment was between 132 and 195. Treatment was significant ($t = 6.09$; df = 9, 15.2; $P \leq 0.001$) with a higher proportion of fungus gnat adults present in the sample compartments containing moist growing medium (45%) compared with those sample compartments containing a dryer sheet and moist growing medium (18%).

Expt. 5: Dryer sheet and growing medium versus dryer sheet and water. The range of fungus gnat adults collected from the colonies and used in the experiment was between 106 and 212. Treatment was not significant ($t = 0.22$, df = 9, 16.2; $P = 0.8271$) with an equal proportion of fungus gnat adults present in the sample compartments containing a dryer sheet and growing medium (15%) as the sample compartments with a dryer sheet and water (15%).

Steam distillation extraction experiment

Analysis of the volatile constituents of the dryer sheets by gas chromatography–mass spectrometry produced a complex chromatogram of which several of the major components were tentatively identified and confirmed by comparison with commercially available

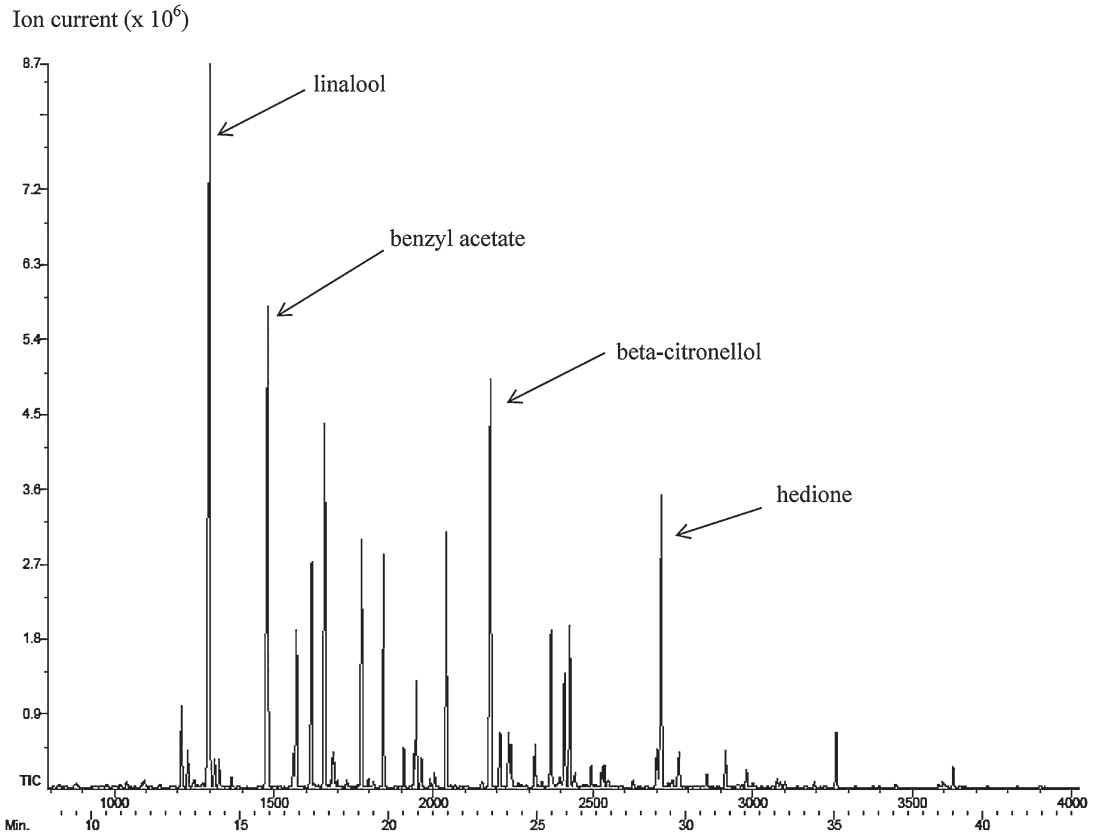

Fig. 2. Total ion chromatogram (gas chromatography–mass spectrometry) of the volatiles detected from each (n = 2) Bounce® original brand fabric softener dryer sheet (Procter and Gamble, Cincinnati, OH).

standard components: linalool, benzyl acetate, beta-citronellol, and hedione (Fig. 2).

Discussion

This study is the first to demonstrate that Bounce® original brand fabric softener dryer sheets repel fungus gnat adults under laboratory conditions. For all five experiments, the mean proportion of fungus gnat adults collected in the sample compartments containing the dryer sheets was significantly fewer ranging from 12% to 18%, whereas those sample compartments without dryer sheets contained 33% to 48% of the fungus gnats released in the arena. Furthermore, for Expts. 1 through 4, the percent of fungus gnat adults recovered from the central compartment was between 36% and 48%; however, the percent of fungus gnats collected from the sample compartment for Expt. 5, which included dryer sheets in both sample compartments, was 69%. This suggests that for Expt. 5, fungus gnat adults remained in the central compartment, further validating evidence for repellent activity of the dryer sheets when placed in both sample compartments. Fungus gnat adults are highly attracted to moist growing medium (Baker 1994; Cloyd et al., 2007b; Olson et al., 2002); however, dryer sheet repellency, as demonstrated in Expts. 3 and 5, clearly overcame any attraction to the moist growing medium.

One of the major volatile constituents detected in the dryer sheets was linalool (3,7-dimethyl-1,6-octadien-3-ol), which is a monoterpene alcohol, colorless liquid that is used by cosmetic and perfume companies as a result of its flower-like odor (Bernier et al., 2007). Linalool is present naturally in plants, including lavender (*Lavandula angustifolia* Mill.), marjoram (*Origanum vulgare* L.), and basil (*Ocimum basilicum* L.) (Mansour et al., 1986; Morales et al., 1993; Narusuye, 2005). This compound occurs in two different structurally active isomer forms chemically known as enantiomers that are chiral molecules having the same molecular formula but are mirror images of each other (Ibrahim et al., 2001). Sometimes, one form is more predominant than the other (Bernier et al., 2007). For example, the major form in lavender is the (R-) form and in coriander (*Coriandrum sativum* L.) the (S+) form (Cseke et al., 2007).

Linalool has been shown to be directly toxic to a number of different mite and insect pests, including *Tyrophagus putrescentiae* (Schrank) (Sanchez-Ramos and Castanera 2001), *Tetranychus cinnabarinus* (Boisd.) (Mansour et al., 1986), *Oryzaephilus surinamensis* (L.) (Shaaya et al., 1991), *Blattella germanica* L. (Jang et al., 2005), and *Zabrotes subfasciatus* (Boheman), *Acanthoscelides obtectus* Say, *Rhyzopertha dominica* (F.), and *Sitophilus oryzae* L. (Weaver et al., 1991). However, minimal research has been conducted to evaluate any repellent activity. It is interesting to note that the citrosa plant (*Pelargonium citrosum* 'Van Leenii'), which is promoted extensively because it claims to repel mosquitoes, contains ≈6.8% linalool (Matsuda et al., 1996).

Citronellol [3, 7-dimethyloct-6-en-1-al ($C_{10}H_{18}O$)] is a monoterpene found in many plants, including rose geranium (*Pelargonium graveolens* L'Her. Ex Ait.), citronella (*Cymbopogon nardus* L.), European pennyroyal (*Mentha pulegium* L.), and lemon balm (*Melissa officinalis* L.). Citronellol is used in sweet lemon scent perfumes and has demonstrated repellent activity against mosquitoes, although the duration of repellency is 1 h or less (Moore et al., 2007).

Based on the results obtained from our study, it is evident that Bounce® original brand fabric softener dryer sheets repel fungus gnats. However, there are still important issues that need to be resolved, including the residual effects (based on age of dryer sheets) and effective distance of repellency, response in a no-choice situation (if dryer sheets are placed into each petri dish), impact on fungus gnat larval populations, and ultimately plant damage. In the future, the use of dryer sheets may be an alternative strategy to deal with fungus gnat populations in greenhouses. This may involve placing individual dryer sheets into a designated number of containers with plants, which would repel adults and prevent egg-laying by females, thus reducing larval populations.

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