Attractiveness of Parboiled Rice Hulls to the Fungus Gnat, Bradysia sp. nr coprophila (Diptera: Sciaridae), Adult Relative to Standard Growing Medium Components

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Abstract
This study was conducted to assess the attractiveness of growing media containing parboiled rice hulls (PBH) to fungus gnat, Bradysia sp. nr. coprophila (Diptera: Sciaridae), adults. In comparing commercially prepared PBH with peatmoss (LC1) and pine bark (SB200)-based growing media, it was established that the fungus gnat adults were not specifically attracted to any of the growing media, even those containing PBH, with the mean proportion of fungus gnat adults recovered in the sample compartments ranging from 0.16 to 0.23. Moisture content was more important in terms of fungus gnat adult attractiveness to the growing media. In addition, the volatile constituents of the various growing media were determined using a steam distillation procedure. The component that was present in the highest concentration (39.2%) in the dried PBH as determined by gas chromatography analysis was palmitic acid, a straight-chain C16 fatty acid. S8 (cyclo-octasulfur), a well-known odoriferous component of degraded waste materials, was present at a higher concentration (6.2%) in the RH1 growing medium (80% peatmoss) compared with the other growing media evaluated. The data indicate that PBH, when incorporated in certain growing media, do not attract fungus gnat adults, and as such, greenhouse producers can use PBH as an amendment to growing medium without having to be concerned with the prospect of luring fungus gnat adults and sustaining plant damage.

Fungus gnats (Bradysia spp.) are common insect pests of greenhouse-grown crops (Dennis, 1978; Hamlen and Mead, 1979). The adults are primarily considered a nuisance causing minimal actual plant damage (Cloyd, 2000), although the eggs laid by adult females hatch into larvae that are directly responsible for causing injury to plants by feeding on the roots (Fawzi and Kelly, 1982; Hungerford, 1916; Jarvis et al., 1993; Kennedy, 1971; Wilkinson and Daugherty, 1970). Growing media may vary in attracting fungus gnat adults (Lindquist, 1994). Meers and Cloyd (2005), for example, reported that female adults of the fungus gnat, Bradysia sp. nr. coprophila Lintner (Diptera: Sciaridae), tend to lay eggs more often in Metro-Mix 560 (The Scott’s Company, Marysville, OH) or Sunshine LC1 Mix (Sungro Horticulture, Inc., Bellevue, WA) soil near composted pine bark (35% to 45%) and coconut coir pith (20% to 30%), whereas the primary components of both SB300 Universal Professional Growing Mix and Sunshine LC1 Mix are composted pine bark (50%) and Canadian sphagnum peatmoss (75%), respectively.

The growing medium type and components may affect the population dynamics of fungus gnats, providing a favorable substrate for development and reproduction (Jagdale et al., 2004). In addition, fungus gnat adults appear to be attracted to or prefer moist growing media containing peatmoss (Baker, 1994). This may provide an abundant level of fungal activity (Freeman, 1983; Olson et al., 2002), which is attractive to and serves as a food source for adult fungus gnats (Anas and Reedeer, 1988; Gardiner et al., 1990; Kennedy, 1974).

Parboiled rice hulls (PBH) are a type of fresh rice hull derived specifically from parboiled rice that are obtained during a steaming process (Evans and Gachukia, 2008). It has been demonstrated that PBH may be an alternative to using perlite, which is an inorganic expanded aluminosilicate byproduct of volcanic origin (Nelson, 2003) incorporated into growing media to improve drainage and air-filled pore space. However, perlite is expensive to mine, transport, and heat. Furthermore, the dust emitted may irritate eyes and lungs (Gachukia and Evans, 2008). Parboiled rice hulls may be included in growing media used for the production of ornamental plants (Evans and Gachukia, 2004). Nonetheless, the potential attractiveness of PBH to fungus gnats is not known. This is important because any growing medium component that attracts fungus gnat adults may lead to significant crop damage as a result of feeding by the larvae. As such, the objective of this study was to determine the attractiveness of PBH to the fungus gnat, Bradysia sp. nr coprophila, adults compared with standard growing medium components under laboratory conditions.

Materials and Methods
A series of seven replicated experiments were conducted to ascertain whether PBH or growing media containing PBH are attractive to fungus gnat adults. Four different growing media were evaluated: Sunshine LC1 Mix (75% sphagnum peatmoss and 25% perlite), RH1 (80% peatmoss and 20% rice hulls), SB200 (60% peatmoss, 20% bark, and 20% perlite), and RH20 (100% rice hulls). The growing media were supplied by SunGro Horticulture, Inc. All experiments were conducted in the absence of a light source 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 in Cloyd et al. (2007b), which involved the use of a set of five six-armed experimental arenas consisting of a central compartment made from a clear, round 5.3-L polypropylene microwavable container (Flex & Seal®; Rubbermaid, Inc., Fairlawn, OH) accompanied by snap-on lids (Fig. 1).

Experimental procedures. Fungus gnat adults used in the study were obtained from laboratory colonies reared on SB300 Universal Professional Growing Mix (Cabreria et al., 2005). Fungus gnat adults used in all the experiments were 6 to 9 d 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 a 9-dram plastic
vial (BioQuip 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.

All experiments were conducted in a laboratory room located in Waters Hall (Kansas State University), Manhattan, KS. The room temperature was 24 ± 3 °C. For the multiple-choice experiments, the experimental arenas were positioned on the floor of the laboratory with the treatments arranged randomly within each experimental arena. For the two-choice experiments, the experimental arenas were placed on a table (1.8 × 0.6 m).

Previous research has demonstrated 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 implement the procedure of releasing the fungus gnat adults into the central compartment); after all the vials had been placed into the central compartments, the door was closed. Yellow sticky cards (2.5 × 2.5 cm) were positioned on the surface of each growing medium. The following experiments were performed in the study: Expt. 1 (rice hull growing medium versus perlite growing medium); Expt. 2 (moist versus moist growing medium—different growing media); Expt. 3 (moist versus moist growing medium—different growing media); Expt. 4 (moist versus moist growing medium—different growing media); Expt. 5 (moistened versus dry growing medium); Expt. 6 (moist versus moist growing medium—different growing media); and Expt. 7 (moist versus dry growing medium—same growing medium).

All experiments were conducted in the dark. 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. In addition, adult fungus gnats flying around within the compartment were collected with use of an aspirator and then the number was recorded.

Steam distillation procedure. The volatile odor constituents of the growing media were determined using a steam distillation procedure (Cloyd et al., 2007b), which produced an extract that was analyzed using gas chromatography. In brief, ≈50 g of material was placed into a Kugelrohr flask (Sigma, St. Louis, MO) that was attached to a condensing column through which steam was passed and collected as a condensed liquid. The aqueous liquid was then extracted with methylene chloride followed by derivatization to form methyl esters of the polar acid compounds. Compound identification was determined by gas chromatography using a mass selective detector and confirmed by authentic standards (Sigma). Quantitative analysis was assessed by gas chromatography with a flame ionization detector.

Data analysis. Data were calculated per replicate as a proportion of 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. All the total number collected using a Statistical Software Program (SAS Systems for Windows, Version 8.2). For Expt. 1, data were normalized by arcsine square root transformation and subject to a one-way analysis of variance with sample compartment as the main effect (SAS Institute, 2002). Significant sample compartment means were separated using a Fisher’s protected least significant difference test at P = 0.05. For Expts. 2 through 7, 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 nontransformed.

Results

Expt. 1: Rice hull growing medium versus perlite growing medium. Treatment was not significant (F = 0.89; df = 3, 39; P = 0.454) as all the treatments (RH20, LC1, RH1, and SB200) had similar proportions of fungus gnat adults present in the sample compartments (Table 1). The range of fungus gnat adults collected from the colonies and used in the experiment was between 90 and 120.

Expt. 2: Moist versus moist growing medium—different growing media. Treatment was significant (t value = 2.61; df = 9, 16; P = 0.019) with a higher proportion of fungus gnat adults present in the sample compartments containing moistened RH20 (41%) compared with moistened SB200 (31%). The range of fungus gnat adults collected from the colonies and used in the experiment was between 75 and 170.

Expt. 3: Moist versus moist growing medium—different growing media. Treatment was not significant (t value = 1.46; df = 9, 13.1; P = 0.168) with a similar proportion of fungus gnat adults present in the sample compartments containing either moistened LC1 (39%) or moistened SB200 (43%). The range of fungus gnat adults collected from the colonies and used in the experiment was between 75 and 130.

Expt. 4: Moist versus moist growing medium—different growing media. Treatment was not significant (t value = 0.75; df = 9, 17.7; P = 0.462) with a similar proportion of fungus gnat adults present in the sample compartments containing either moistened RH1 (36%) or moistened SB200 (41%). The range of fungus gnat adults collected from the colonies and used in the experiment was between 95 and 160.

Expt. 5: Moistened versus dry growing medium. Treatment was significant (t value = 6.76; df = 9, 16; P < 0.0001) with a higher proportion of fungus gnat adults present in
the sample compartments containing moistened SB200 (52%) than sample compartments containing dry RH20 (17%). The range of fungus gnat adults collected from the colonies and used in the experiment was between 80 and 140.

Expt. 6: Moist versus moist growing medium—different growing media. Treatment was not significant (t value = 0.32; df = 9, 17; P = 0.750) with a similar proportion of fungus gnat adults present in the sample compartments containing either moistened RH1 (34%) or moistened LC1 (32%). The range of fungus gnat adults collected from the colonies and used in the experiment was between 100 and 170.

Expt. 7: Moist versus moist growing medium—same growing medium. Treatment was significant (t value = 4.57, df = 9, 17; P = 0.003) with a higher proportion of fungus gnat adults present in the sample compartments containing moistened RH20 (53%) than sample compartments containing dry RH20 (17%). The range of fungus gnat adults collected from the colonies and used in the experiment was between 110 and 180.

Steam distillation procedure. The rice hull material had very little, if any, discernible odor. RH1 had an odor similar to ordinary peatmoss. LC1, which contains \( \approx 75\% \) sphagnum peatmoss, had more of a “mushroom”-like odor and the growing medium SB200 had an “earthy” odor. In general, the aqueous steam distillate of each of these materials had an odor quite similar to “peatmoss.”

Results of the gas chromatography–flame ionization detector analysis are presented in Table 2. The major components identified were very similar to the types of compounds previously identified from a commercial peatmoss material (Cloyd et al., 2007b). Those components that were present included the fatty acids, C6-C18, and the dicarboxylic acids, C4-C5 as well as furfural and 5-methylfurifural. Cyclosulfur (S8) was present in each of the commercially mixed growing media, but not in the rice hull material. The rice hull material contained nearly 10 times as much C16 (39.2%) as the other growing media tested (Table 2).

Discussion

This study demonstrated that PBH are not attractive to fungus gnat adults. What appears to be most important in terms of fungus gnat adult attractiveness is the growing medium moisture content. In our study, fungus gnat adults were attracted to moist growing media and were less attracted to growing media with low moisture contents (less than 10%), which may be associated with survival of fungus gnat eggs and larvae (Cloyd and Dickinson, 2008; Ellisor, 1934). Furthermore, Olson et al. (2002) determined that fungus gnat larval survival was higher in a peat-based growing medium (35% to 45% sphagnum peatmoss) with a moisture content between 52% and 71% compared with growing media with a moisture content less than 34% and maintained at 90%.

The growing media RH1 and RH20 are blends from a commercial vendor (SunGro Horticulture, Inc.) with 20% and 100% PBH, respectively. Although the RH20 growing medium is supposed to contain 100% PBH, there were trace amounts of peatmoss present, enough so that a “characteristic” peatmoss-like odor was detectable before analysis. Consequently, a purer sample of PBH, with no peatmoss present, was obtained from the vendor. There was no discernible odor from this new material. The most abundant constituent that was present in the highest concentration in the dried PBH as determined by gas chromatography analysis was n-C16 (palmitic acid). Palmitic acid, a saturated straight-chain acid, is commonly present in plant material. The higher concentrations obtained in the rice hulls most likely indicate that this material has not undergone sufficient physical decay with the hulls still intact. In materials such as peatmoss or composted bark that are exposed to a specific degradation treatment, the long chain fatty acids are fragmented into shorter chains or more oxidized components.

Previous studies have demonstrated that crop residues from rice fields or composted rice hulls contain numerous secondary metabolites (see review by Rimando and Duke, 2003). The PBH used in this study and subsequent experiments were cleaned and subjected to heat by the manufacturer, which is similar to treatments that produce composted bark, thus providing a material that may be incorporated into growing medium. To assess the odoriferous components of growing media, which may act as lures for fungus gnat colonization, this study focused on the volatile constituents. Aqueous or organic solvent extracts of rice residue (or any plant residue) should yield the very complex phenolics, terpenes, and steroid constituents, which have been identified in rice (Oryza sativa L.). However, none of these compounds were detected in the collected steam distillate. Instead, fatty acids, primarily palmitic (C16) and stearic (C18) acid, were the main constituents with small amounts of shorter-chain fatty acids and diacids (Table 2). Minimal quantities of furfural and methylfurfural as well as dodecanol were also detected. Both the RH1 and RH20 material had similar profiles; however, additional volatiles were also present, which

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Table 1. Mean (± SEM) proportion of fungus gnat, Bradysia sp. nr. coprophila, adults recovered in the sample compartments associated with each experimental arena for each growing medium treatment in Expt. 1 (n = number of replications per treatment).

<table>
<thead>
<tr>
<th>Treatment (growing medium)</th>
<th>n</th>
<th>Mean (± SEM) proportion fungus gnat adults recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>RH20</td>
<td>10</td>
<td>0.21 ± 0.03 a*</td>
</tr>
<tr>
<td>LC1</td>
<td>10</td>
<td>0.22 ± 0.03 a</td>
</tr>
<tr>
<td>RH1</td>
<td>10</td>
<td>0.23 ± 0.03 a</td>
</tr>
<tr>
<td>SB200</td>
<td>10</td>
<td>0.16 ± 0.02 a</td>
</tr>
</tbody>
</table>

*Means followed by common letter are not significantly different based on a Fisher’s protected least significant difference test with \( P = 0.05 \).

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Table 2. Percent composition of volatiles detected in rice hulls and growing media using steam distillation procedure as determined by gas chromatography (GC) analysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rice hulls (dry)</th>
<th>RH1 (dry)</th>
<th>RH20 (dry)</th>
<th>LC1 (dry)</th>
<th>SB200 universal mix (dry)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicarboxylic acids*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>0.2</td>
<td>0.3</td>
<td>1.5</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>C5</td>
<td>0.1</td>
<td>0.4</td>
<td>1.4</td>
<td>0.9</td>
<td>1.3</td>
</tr>
<tr>
<td>C6</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>C7</td>
<td>NP</td>
<td>NP</td>
<td>0.1</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>C8</td>
<td>NP</td>
<td>0.1</td>
<td>0.6</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Alkanoic fatty acids*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>1.9</td>
<td>0.6</td>
<td>0.3</td>
<td>NP</td>
<td>0.8</td>
</tr>
<tr>
<td>C7</td>
<td>0.4</td>
<td>0.6</td>
<td>0.1</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>C8</td>
<td>2.5</td>
<td>0.6</td>
<td>1.2</td>
<td>5.3</td>
<td>3.8</td>
</tr>
<tr>
<td>C9</td>
<td>1.8</td>
<td>0.6</td>
<td>0.1</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>C10</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>C11</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>NP</td>
</tr>
<tr>
<td>C12</td>
<td>1.7</td>
<td>1.5</td>
<td>0.7</td>
<td>1.1</td>
<td>NP</td>
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<tr>
<td>C14</td>
<td>3.1</td>
<td>0.3</td>
<td>1.7</td>
<td>0.7</td>
<td>0.6</td>
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<tr>
<td>C16</td>
<td>39.2</td>
<td>2.0</td>
<td>7.8</td>
<td>1.0</td>
<td>2.9</td>
</tr>
<tr>
<td>C18</td>
<td>1.3</td>
<td>0.8</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
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<tr>
<td>Furfural</td>
<td>0.2</td>
<td>2.8</td>
<td>NP</td>
<td>10.1</td>
<td>3.9</td>
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<tr>
<td>Methyl furfural</td>
<td>0.2</td>
<td>1.5</td>
<td>NP</td>
<td>2.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Camphor</td>
<td>NP</td>
<td>0.1</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Verbenone</td>
<td>NP</td>
<td>0.5</td>
<td>NP</td>
<td>NP</td>
<td>0.4</td>
</tr>
<tr>
<td>1,3-dimethoxybenzene</td>
<td></td>
<td>0.1</td>
<td>NP</td>
<td>NP</td>
<td>0.4</td>
</tr>
<tr>
<td>Dodecanol</td>
<td>0.2</td>
<td>2.3</td>
<td>0.9</td>
<td>1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>Cyclosulfur (S8)</td>
<td>NP</td>
<td>6.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

* Determined as methyl ester.
NP = not present based on gas chromatography–flame ionization detector or gas chromatography–mass selective detector.

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were detected in the LC1 and SB200 material. Furthermore, cyclosulfur (S8) was detected in each of the commercially available growing media with the highest amount in the RH1 growing medium (80% peatmoss).

A number of analytical techniques are available to characterize odor profiles. Steam distillation has proven to be sufficient in obtaining samples from various soilless growing media that contain a range of plant-based constituents such as terpenes and phenolics as well as the volatile cellular breakdown products such as diacids and fatty acids. Previous research with peatmoss and composted pine bark has demonstrated that the C6-C18 fatty acids are more prevalent in peatmoss material (Cloyd et al., 2007b). In the previous research as well as our current study, the more pungent acids such as propionic or butyric acid were not present.

Evidence of microbial transformation in the composted materials can also be revealed in these volatile analyses. Composted pine bark would be expected to contain pinene and camphene, but instead borneol and alpha-terpineol, which are both microbial metabolites of the two terpenes (Braddock and Cadwallader, 1995), were present. Cyclosulfur (S8) was detected in each of the materials that contained peatmoss: RH1, RH20, LC1, and SB200. This pungent off-odor compound may be formed by bacterial oxidation of hydrogen sulfide (Steudel, 1996). Experimental arenas such as the one used in our study are a common method to quantify the attraction of compounds to insects under laboratory conditions (Gothilf and Galun, 1982; Martin et al., 1990). Moreover, it was essential to conduct all the experiments in the laboratory so as to determine insect responses and eliminate any unknown variables or confounding factors that could have occurred under field conditions. We also used types of plastic (polypropylene and acrylic) that do not emit detectable volatiles, which could have influenced our results.

This study was designed to assess the attractiveness of PBH when incorporated in growing media to fungus gnat adults. As such, we have demonstrated that Bradysia sp. nr. coprophila adults do not appear to prefer PBH when compared with other growing medium components. This indicates that greenhouse producers may use PBH as an amendment to growing medium without having to be concerned with the prospect of luring fungus gnat adults and sustaining plant damage.

**Literature Cited**


