Effects of Growing Medium Type and Moisture Level on Predation by Adult Rove Beetle, Dalotia coriaria (Coleoptera: Staphylinidae), on Fungus Gnat, Bradysia sp. nr. coprophila (Diptera: Sciaridae), Larvae under Laboratory and Greenhouse Conditions

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Abstract. The fungus gnat, Bradysia sp. nr. coprophila (Lintner) (Diptera: Sciaridae), is an insect pest of greenhouse production systems. The rove beetle, Dalotia coriaria [Kraatz] (Coleoptera: Staphylinidae), is a commercially available predator of certain greenhouse insect pests that reside in growing media, including fungus gnats. There is minimal information discussing how growing medium type and moisture level (watering treatment) impact the interactions between pests and natural enemies. Therefore, we conducted laboratory and greenhouse experiments to investigate the influence of two growing media (Sunshine® LC1 Professional Growing Mix and Fafard® 3B Mix Professional Formula) and two moisture levels (“constantly saturated” and “initially saturated”) on predation by adult D. coriaria on B. sp. nr. coprophila larvae after releasing one or two rove beetle adults. In the laboratory experiment, moisture content or the amount of water retained by the growing medium did not significantly influence the recovery of adult fungus gnats for any of the rove beetle treatments. However, there was a significant difference in the recovery of fungus gnat adults between the two growing media. Fewer fungus gnat adults emerged from the Sunshine® LC1 Professional Growing Mix (0.9 ± 0.2 adults) than the Fafard® 3B Mix Professional Formula (6.0 ± 0.9 adults). Significantly fewer adult fungus gnats were recovered in the treatments where one rove beetle adult was released (2.7 ± 0.7 adults) and two rove beetle adults were released (2.3 ± 0.5 adults) compared with the control without rove beetles (5.4 ± 1.4 adults). However, there was no significant difference between the number of rove beetle adults released. In contrast to the laboratory experiment, moisture content in the greenhouse experiment significantly influenced the recovery of adult fungus gnats. More adult fungus gnats were recovered from the “constantly saturated” treatment (9.9 ± 1.4 adults) than the “initially saturated” treatment (3.8 ± 1.0 adults). Similar to the laboratory experiment, there was a significant difference in the recovery of fungus gnat adults between the two growing media, with fewer adults captured from the Sunshine® LC1 Professional Growing Mix (3.2 ± 0.8 adults) than the Fafard® 3B Mix Professional Formula (10.4 ± 1.4 adults). However, the treatments with rove beetle adults [one rove beetle (6.6 ± 1.8 adults) or two rove beetles (5.3 ± 1.5 adults)] were not significantly different from the control without rove beetles (8.6 ± 1.5 adults), suggesting that the growing media and moisture levels were acting directly on fungus gnat survival. The results of our study demonstrate that survival of fungus gnat larvae that reside in the growing medium and the success of rove beetle adults used to regulate these pests can be influenced by growing media and the moisture content within growing media.

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Materials and Methods
The following study consisted of laboratory and greenhouse experiments designed to assess whether common greenhouse horticultural practices, such as growing medium and irrigation, influence the ability of rove beetles to regulate fungus gnat larval populations.

Laboratory experiment. A laboratory colony of the fungus gnat, B. sp. nr. coprophila (Lintner), was maintained in 8.0-L plastic containers with tight-sealing lids that had openings cut into the lids (11.5 × 22.5 cm) with insect screening (0.2 × 0.8 mm Green-tech®, Edgerton, WI) hot-glued to the lids to...
allow for ventilation. Using a 6.0-L container, growing medium (Sunshine® LC1 Professional Growing Mix; Sun Gro Horticulture, Inc., Bellevue, WA) composed of 70% to 80% Canadian sphagnum peatmoss, perlite, dolomitic limestone, and silicone dioxide was moistened with ≈1.4 L of water, pasteurized in a microwave (Panasonic® Inverter; Panasonic Consumer Electronics Comp., Newark, NJ) at 1250 W for 20 min, and then allowed to cool. Two tubers (240 g each) of potato (Solanum tuberosum L.) were pureed into small particles using a food processor and water (125 mL) and then uniformly mixed into the growing medium by hand. About 3.0 L of the growing medium and potato mixture were placed inside the 8.0-L container. Sixty grams of oats (Avena sativa L.) (The Quaker Oats Company; Chicago, IL) were placed into two piles positioned in opposite corners of the rectangular container on the growing medium surface. The oats were initially moistened with 40 mL of tap water using a 946-mL spray bottle (Spray-Master®, Delta Industries™, King of Prussia, PA). Afterward, the growing medium in the container was moistened daily. About 500 adult fungus gnats (± 4 d postemergence) were aspirated into a 9-dram (33.4 mL) plastic vial from the main colony and added to the container for oviposition (egg-laying) by mated females. The female: male sex ratio of the colony is 9:1 (R.A. Cloyd, unpublished data). Fungus gnat adults were added to the container for 2 to 3 d. Colonies were maintained at 25 ± 5 °C, 50% to 60% relative humidity (RH), and constant light under laboratory conditions in the Department of Entomology at Kansas State University (Manhattan, KS).

To isolate second and third instar fungus gnat larvae used in the experiment, a glass petri dish (100 × 15 mm) was lined with 9-cm diameter P8 Fisherbrand® filter paper (Thermo Fisher Scientific, Pittsburgh, PA) and placed into a 750-mL food storage container (Ziploc®; SC Johnson; Racine, WI). About 9.5 g of growing medium with pureed potato was placed on top of the filter paper and moistened with a spray bottle, avoiding any standing water. The lid was placed onto the storage container; ≈500 adults were collected from the main colony and then released into the containers so that mated females could lay eggs. After 10 to 11 d, second and third instar larvae were available for use in the experiment.

The rove beetle, D. coriaria [Kraatz], used in the experiment was maintained similarly to the fungus gnats; however, no potato was added to the growing medium and 200 g of oats were placed in the center of the container on the growing medium surface and moistened with 130 mL of water using a plastic spray bottle. The colony was maintained at 25 ± 5 °C, 50% to 60% RH, and under constant darkness.

The laboratory experiment was performed in the Horticultural Entomology and Plant Protection Laboratory in the Department of Entomology at Kansas State University. Using a glass pipette, 20 second to third instar fungus gnat larvae were placed into 473-mL deli containers (Fabri-Kal Corp.; Kalamazoo, MI) with ≈300 mL of growing medium, prepared as described above; however, the growing medium did not contain any potato or oats. Then, 150 mL of water was added to achieve 100% container capacity after placing the growing medium into the deli containers. One-hundred percent container capacity was obtained based on observations of water leaching through the bottom of the deli containers, indicating that the growing medium was no longer capable of retaining additional water. The lids of the deli containers were modified with inset screening (0.2 × 0.8 mm) to allow for ventilation, and 12 holes were perforated into the bottom using a dissecting probe. The bottoms were covered with insect screening (0.2 × 0.8 mm) to prevent larvae from escaping through the holes. The holes in the bottom allowed for any excess water to drain or for reabsorption by the growing medium.

Each deli container was placed into a plastic holding dish (1.5 × 14 cm) and maintained at 25 ± 5 °C, 50% to 60% RH, and a 16:8 (light:dark) hour photoperiod. Fungus gnat larvae were allowed to disperse undisturbed within the growing medium for 24 h (Cabrera et al., 2003). Afterward, one or two rove beetle adults (2 to 4 d postemergence) were added to the designated treatments, except for the control without rove beetles. There were two growing medium types used: Sunshine® LC1 Professional Growing Mix (Sun Gro Horticulture, Inc., Bellevue, WA) composed of 70% to 80% Canadian sphagnum peatmoss, perlite, dolomitic limestone, and silicone dioxide; and Fafard® 3B Mix Professional Formula (Sun Gro Horticulture Canada, Ltd., Seba Beach, Alberta, Canada) composed of 50% to 60% Canadian sphagnum peatmoss, composted pine bark, perlite, vermiculite, and dolomitic limestone. Herein, the two growing media will be referred to as Sunshine® LC1 Mix and Fafard® 3B Mix. There were two moisture levels, based on two watering treatments: “constantly saturated” and “initially saturated.” The “constantly saturated” treatment was maintained at 100% moisture content (container capacity), whereas the “initially saturated” treatment allowed for water loss during the course of the experiment. There were 12 treatments with 5 replications per treatment (Table 1).

Rove beetle adults (2 to 4 d postemergence) were removed directly from the colony to simulate conditions that would occur if a greenhouse producer had ordered rove beetle larvae from a biological control supplier. The rove beetle sex ratio was 1:1 (female: male) as reported by Echegaray and Cloyd (2013). The “constantly saturated” treatments were maintained by adding 60 mL of water to each holding dish that each deli container was placed in before complete evaporation or absorption of water occurred (every 3 to 5 d). The “initially saturated” treatments were maintained by withholding any additional water. These moisture levels were chosen to simulate overwatering (“constantly saturated”) and underwatering (“initially saturated”) that are known to occur during greenhouse crop production (Nelson, 2012). Daily weight measurements were recorded, using a scale (Model No. APX-602; Denver Instrument Inc., Bohemia, NY), on the “initially saturated” treatments to obtain percent moisture content estimates over the course of the experiment. Daily measurements were not recorded for the “constantly saturated” treatments because 100% container capacity was maintained. The percent moisture content was calculated by using the following equation modified from Cloyd and Dickinson (2008):

\[
100 - \left(\frac{B - C_{\text{B}}}{A - C_{\text{B}}}\right) \times 100
\]

A = initial weight (g) (deli container + lid + sticky card + petri dish + moist growing medium),
B = weight (g) of deli container + lid + sticky card + petri dish, and
C = daily weight (g) (deli container + lid + sticky card + petri dish + moist growing medium).

After 12 d, a 5 × 4 cm section of a yellow sticky card (Humnett International, Topeka, KS), with only one side of the film removed to expose the sticky side, was carefully placed into each deli container with the sticky surface facing upward and at a 45° angle to the side of the container and growing medium. After 35 d, each yellow sticky card section was inspected, and the number of fungus gnat adults captured was recorded. The use of the yellow sticky card section was an indirect assessment of fungus gnat larval mortality (Cloyd and Dickinson, 2008).

**Greenhouse experiment.** A similar experiment with the same treatments (Table 1) was conducted from 27 Apr. to 31 May 2016, in three separate, but adjacent, greenhouses at Kansas State University to simulate conditions in a commercial greenhouse. The two moisture levels (watering treatments) and the controls were separated among the three greenhouses, and the treatments were randomized within each greenhouse to avoid cross-contamination among the treatments. While rove beetle adults tend to remain in the growing medium after release, we wanted to eliminate the possibility of rove beetle adults migrating into the controls. The environmental conditions of the three greenhouses were the same (temperature: 23 ± 5 °C and RH: 80%).

Coleus [Solenostemon scutellarioides (L.) Codd. cv. Big Red Judy] plants were purchased as plugs from Proven Winners, LLC (Sycamore, IL) and transplanted into 15.2-cm diameter containers. Thirty plugs were transplanted into containers with Sunshine®
LC1 Mix, and another thirty plugs were transplanted into containers with Fafard® 3B Mix.

All 60 coleus plants were labeled according to the appropriate treatment (Table 1), randomly arranged on a wire-mesh bench 20 cm apart, allowed to establish (root system had reached the inner portion of the 15.2-cm diameter container) for 35 d, and were irrigated with 500 mL of water every 2 d. Then on day 1, each container with a plant was placed into a 20-cm diameter plastic holding dish and weighed on a scale (Model MBS-6000; Salter Brecknell®, Fairmont, MN) to establish estimates for the “constantly saturated” and “initially saturated” treatments. The container capacity was established by adding 500 mL of water to each container and waiting until water leached into the holding dish. The “constantly saturated” treatments were provided with 500 mL of water every 2 to 3 d to maintain 100% container capacity. The “initially saturated” treatments were not watered again but were weighed daily to obtain daily percent moisture content estimates. The percent moisture content was calculated as described above, taking into account the container and holding dish weights, as well as the excised plants (stems and leaves) were excised at the base just above the growing medium, weighed, and discarded. A 5 x 4 cm section of a yellow sticky card was weighed and placed on the growing medium surface to capture emerging fungus gnat adults. The containers with growing medium and yellow sticky cards were placed into 3.8-L Ziploc® bags, which were used to prevent adult fungus gnats from escaping. The Ziploc® bags were also weighed. Three 2.5-cm2 holes were cut into the bottom of each Ziploc® bag for the “constantly saturated” treatments. Also, 30 holes were made with a probing needle in the top of each Ziploc® bag to allow for ventilation and reduce condensation. After 35 d, the number of adult fungus gnats captured on each yellow sticky card was recorded. In addition, each Ziploc® bag was rinsed with water into a white 10-L plastic container to account for any fungus gnat adults that adhered to any condensate. The rate of water loss for all treatments was minimal after the plants were excised and when the containers were placed into the Ziploc® bags on day 14 for the “initially saturated” treatments. Therefore, the rate of water loss data was only analyzed when plants were present.

**Experimental design and statistical analysis.** Each experiment was conducted as a 2 x 2 factorial design (growing media, moisture content, and number of rove beetle adults—0, 1, and 2), with five replications associated with each treatment combination. The data for each experiment (laboratory and greenhouse) conformed to the assumptions of an analysis of variance (ANOVA), based on normality and homogeneity of variances (Little and Hills, 1978). Data were normally distributed so no transformation procedure was required. Therefore, the number of adult fungus gnats captured on the yellow sticky cards was analyzed based on an ANOVA (an = 0.05) using PROC GLM (because estimates were not measured overtime), and the rate of moisture loss and percent moisture content estimates were assessed as repeated measures using PROC GLMMIX (SAS Institute, 2002). Individual treatment means associated with adult fungus gnat counts on yellow sticky cards were separated using the least significant difference (LSD) test when the ANOVA indicated a significant treatment effect (SAS Institute, 2002). Plant growth and weight data were analyzed using the ANOVA in PROC GLM with the significant means for the two growing media separated using an LSD test (SAS Institute, 2002).

### Results

**Laboratory experiment.** Rove beetle adult activity was observed throughout the experiment to ensure that adult rove beetles were actively searching for fungus gnat larvae. There was no significant difference (F = 0.10; df = 1, 73; P = 0.75) in the rate of water loss between the Sunshine® LC1 Mix (2.08 ± 0.13 g/d; mean ± SE) and the Fafard® 3B Mix (2.08 ± 0.13 g/d) in the “initially saturated” treatments despite differences in the composition of the two growing media (see Materials and Methods). There was a significant percent water loss over time in the “initially saturated” treatments (F = 241.83; df = 33, 73; P < 0.0001). However, the percent moisture content was similar for all treatments throughout the experiment because of equivalent rates of water loss between the two growing media, and water loss occurred at a greater rate between days 0 and 5 then afterward (Fig. 1).

Moisture level (“constantly saturated” or “initially saturated”) did not significantly influence the recovery of adult fungus gnats (F = 1; df = 1, 10; P = 0.27). However, there was a significant difference in fungus gnat adult recovery between the two growing media (F = 36.26; df = 1, 10; P < 0.0001). Fewer fungus gnat adults were captured on yellow sticky cards affiliated with the Sunshine® LC1 Mix (0.9 ± 0.2 adults) than the Fafard® 3B Mix (6.0 ± 0.9 adults) growing medium. In addition, the number of rove beetle adults released affected the fungus gnat populations with significantly (F = 5.22; df = 2, 10; P = 0.0088) fewer adult fungus gnats recovered in the treatments where one rove beetle adult was released (2.7 ± 0.7 adults) or two rove beetle adults were released (2.3 ± 0.5 adults) compared with the control without rove beetles (5.4 ± 1.4 adults).
adults). However, there was no significant difference in fungus gnat adult recovery between the number of rove beetle adults released (1 or 2) despite a significant interaction between growing media and number of adult rove beetle releases (F = 85.29; df = 2, 10; P = 0.037). There were no significant three-way interactions (P > 0.05).

**Greenhouse experiment.** Rove beetle adult activity was observed throughout the experiment to ensure that adult rove beetles were actively searching for fungus gnat larvae. There was a significant difference (F = 57.48; df = 1, 35; P < 0.0001) in the rate of water loss between the Sunshine® LC1 Mix (51.7 ± 2.3 g/d) and the Fafard® 3B Mix (62.9 ± 2.8 g/d) in the “initially saturated” treatments. There was also a significant percent water loss over time in the “initially saturated” treatments (F = 158.89; df = 14, 35; P < 0.0001). Percent moisture content was similar for all treatments throughout the experiment with equivalent rates of water loss between the two growing media, and water loss occurred at a greater rate between days 0 and 3 then afterward (Fig. 2).

Moisture content significantly influenced the recovery of adult fungus gnats in the greenhouse experiment (F = 19.40; df = 1, 8; P < 0.0001). Percent moisture content fluctuated from initially 100%, to 30% after 36 d across the six “initially saturated” treatments. More adult fungus gnats were recovered from the “constantly saturated” treatments (9.9 ± 1.4 adults) than the “initially saturated” treatments (3.8 ± 1.0 adults) (Fig. 3). Similar to the laboratory experiment, within each moisture level, there was a significant difference associated with the recovery of adult fungus gnats between the Sunshine® LC1 Mix and Fafard® 3B Mix (F = 27.28; df = 1, 8; P < 0.0001) growing media. For example, significantly more fungus gnat adults were recovered from the Fafard® 3B Mix than the Sunshine® LC1 Mix (Fig. 3). In contrast to the laboratory experiment, the treatments with 1 (6.6 ± 1.8 adults) or 2 (5.3 ± 1.5 adults) rove beetles were not significantly different (F = 1.93; df = 1, 8; P = 0.15) from controls without rove beetles (8.6 ± 1.5 adults). In addition, there were no significant two- or three-way interactions (P > 0.05).

There was a significant difference in plant height (F = 34.16; df = 1, 7; P < 0.0001). Plants growing in the Sunshine® LC1 Mix were shorter (10.4 ± 0.5 cm) than plants growing in the Fafard® 3B Mix (14.4 ± 0.5 cm). Furthermore, plant fresh weight was significantly less (F = 92.59; df = 1, 6; P < 0.0001) for plants growing in the Sunshine® LC1 Mix (19.9 ± 2.1 g) compared with plants growing in the Fafard® 3B Mix (42.6 ± 4.4 g).

**Discussion**

This is the first study to investigate how different growing medium types and moisture levels affect predation of fungus gnat larvae by *D. coriaria* adults. Cultural practices associated with greenhouse crops production are typically not taken into consideration when implementing biological control programs. Both growing medium type and moisture level had an impact on fungus gnats, either directly or indirectly via effects on the predator, *D. coriaria*. However, results were not consistent between the laboratory and greenhouse experiments. The differences in moisture contents for the laboratory and greenhouse experiments were probably due to the rate of water loss for all the treatments. The rate of water loss was delayed in the laboratory experiment under controlled conditions, and allowed for a longer period of water retention, which likely aided fungus gnat larval survival and contributed to the lack of moisture content having a direct effect on the recovery of fungus gnat adults. However, this was not the case in the greenhouse experiment. The differences in water loss between the laboratory and greenhouse experiments were likely due to the presence of plants. The lack of moisture in the “initially saturated” treatment impacted the survival of fungus gnat larvae. Clowd and Dickinson (2008) reported the highest fungus gnat larval survival at moisture contents between 56 and 68%. In our study, moisture contents were lower than 56% (as low as 30%) and higher than 68% (as high as 80%), which influenced the survival of fungus gnat larvae.

In the laboratory experiment, there was a significant interaction between the two growing media and the number of rove beetle adults released (1 or 2), with adult rove beetles significantly reducing fungus gnat adult captures on the yellow sticky cards in relation to the initial larval inoculations compared with the control without rove beetles, suggesting that changes in fungus gnat numbers are dependent on each effect (the growing medium and the number of rove beetle adults released). However, a similar effect was not apparent in the greenhouse experiment where there was no evidence that rove beetles reduced fungus gnat larval populations compared with the control without rove beetles.

The rove beetles may not have reduced fungus gnat adult captures in the greenhouse experiment because of the increased searching environment (Echegaray et al., 2015). Consequently, the higher volume and amount of growing medium in the 15.2 cm diameter containers (1834.82 cm³ and 2.0 L of growing medium) compared with the smaller searching area and less volume and amount of growing medium in the deli containers (616.14 cm³ and 0.3 L of growing medium) likely inhibited the ability of rove beetles to locate fungus gnat larvae. In addition, fungus gnat larvae may have had more areas to hide; thus escaping exposure from rove beetle adults (Echegaray et al., 2015). Therefore, results from the current study indicate that future studies are warranted to investigate releasing rove beetles at different numbers, based on container size, which would be more applicable under greenhouse production systems.

In our study, growing medium type affected the recovery of fungus gnat adults in both the laboratory and greenhouse experiments. Growing medium can directly or indirectly impact fungus gnat larval survival or affect rove beetle predation (Echegaray et al., 2015; Lindquist et al., 1985; Pacchioli and Hower, 2004). We hypothesize that the composition of the growing medium (based on the overall components) may have impacted the interaction between fungus gnat larvae and rove beetle adults. For example, the micropores and macropores associated with
the growing medium in conjunction with moisture content may have affected survival and movement of both fungus gnat larvae and rove beetle adults (Collis-George, 1959; Strnad and Bergman, 1987; Villani and Wright, 1990). Moreover, the movement of rove beetle adults may have been impacted by growing medium type (Pacchioli and Hower, 2004), which could have influenced predation. Studies have indicated that insect movement can be inhibited by soil structure, pore size distribution, and moisture content (Gustin and Schumacher, 1989; MacDonald and Ellis, 1990).

During the course of the study, we observed a noticeable difference in plant height of the coleus plants affiliated with the two growing media. We suspect that the plant height and fresh weight differences were a consequence of the variability in the composition of the growing media. For instance, the silicone dioxide in the Sunshine® LC1 Mix that was not a component of the Fafard® 3B Mix may have been harmful to the coleus plants although the concentration was only 0.001%. The Fafard® 3B Mix included composted pine bark and vermiculite, which were not a component of the Sunshine® LC1 Mix. However, composted pine bark and vermiculite should not harm plants since they are main components of many commercially available growing media. The fungus gnat larval populations were lower, and the coleus plants were significantly more stunted in the Sunshine® LC1 Mix than the Fafard® 3B Mix. Another aspect of our study is that the Sunshine® LC1 Mix, which had the fewest fungus gnat adults captured on the yellow sticky cards, also negatively influenced the growth of the coleus plants compared with plants grown in the Fafard® 3B Mix.

The effect on plant growth that we observed may be affiliated with fewer micro- pores and macropores, thus reducing the ability of the roots to uptake water and nutrients in the Sunshine® LC1 Mix. Riaz et al. (2008) reported that growing medium type affected the growth of Zinnia elegans Jacq. plants. So, what we observed in our study, in terms of the influence of growing medium on plant growth, may occur in the greenhouse industry unbeknownst to greenhouse producers. Therefore, future research efforts should concentrate on determining if growing medium type, including chemical composition and physical characteristics, is responsible for any differences in plant height and fresh weight of horticultural crops grown in greenhouses, which could impact (delay) production scheduling.

The lack of efficacy associated with the number of rove beetle adults released (1 or 2) may be a consequence of the greater searching area (based on container volume) of the 15.2-cm diameter plastic containers used in the greenhouse experiment (1834.82 cm³ with 2.0 L of growing medium) compared with the smaller searching area of the deli containers used in the laboratory experiment (616.14 cm³ with 0.3 L of growing medium). However, before conducting our study, we were not aware of nor is there information available indicating that searching area may influence the efficacy of soil-inhabiting predators including rove beetles other than that proposed by Echegaray et al. (2015). Moreover, further investigations are needed to determine if using more rove beetles would enhance efficacy against fungus gnat larvae in larger containers. Therefore, the impact of cultural practices, such as growing medium type and moisture level (watering treatment), should be taken into consideration when implementing biological control programs in greenhouse production systems.
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