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Host-associated volatiles attract parasitoids of a native solitary bee, *Osmia lignaria* Say (Hymenoptera, Megachilidae)

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Abstract

Parasitoids use volatiles to recognize and locate suitable hosts. Numerous studies have investigated parasitoid host location from a pest management perspective, but comparatively little is known regarding parasitoid-pollinator interactions. Previous research has shown that parasitoids of some native bees respond to volatiles emitted by host frass and cocoons. We used a Y-tube bioassay to test whether two parasitoid species (*Monodontomerus torchioi* and *Melittobia acasta*) are attracted to volatiles associated with their host, the blue orchard bee, *Osmia lignaria*. Specifically, we tested attraction to (1) cocooned adult *O. lignaria* females (2) *O. lignaria* frass (3) methanol and (4) acetic acid. Both parasitoid species were attracted to host frass and acetic acid. Although *M. acasta* showed a strong attraction to volatiles from cocooned *O. lignaria* females, it was repelled by methanol, a chemical constituent of *Osmia* cocoons. In contrast, *M. torchioi* showed no response to cocooned *O. lignaria* and only a slight attraction to methanol. Both parasitoid species appear to be differentially attracted to specific host-associated volatiles, suggesting that they may respond to distinct olfactory cues when locating potential bee hosts.

Keywords

Y-tube bioassay, olfactory cues, solitary bee, Megachilidae, *Melittobia acasta*, *Monodontomerus torchioi*

Introduction

Parasitoids use a variety of chemical cues to identify and locate potential hosts, including those emitted by the host's habitat (Vet et al. 1984), host byproducts (i.e. frass, Agelopoulos et al. 1995), organisms living in association with the host (Sullivan et al. 2000), and the host itself (Wiskerke et al. 1992, Jumean et al. 2009). Numerous studies have investigated parasitoid attraction to host-associated volatiles in agroecosystems, often with the goal of developing more effective pest management strategies (Jones et al. 1973, Godfray 1994, Jumean et al. 2009). However, despite the ecological and economic importance of pollinators, few studies have investigated how parasitoids recognize and locate their bee hosts (but see Silva-Torres et al. 2005, Filella et al. 2011). A number of solitary bee species are currently used to pollinate commercial crops, including *Nomia melanderi* Cockrell (Halictidae) and *Megachile rotundata* Fabricius (Megachilidae) in alfalfa (Kemp and Bosch 2000, Cane 2008) and *Osmia* spp. (Megachilidae) in almonds (Bosch and Kemp 2001). Many solitary bee species are attacked by a diversity of hymenopteran parasitoids and brood parasites during development, sometimes resulting in high rates of mortality prior to adult eclosion (Vicens et al. 1994, Bosch and Kemp 2001, Wcislo and Cane 1996). Thus, building a more robust understanding of parasitoid host location is not only of interest from an ecological perspective, it may also aid efforts to control parasitism in commercially important pollinator species.

The blue orchard bee (*Osmia lignaria* Say, Megachilidae) is a univoltine solitary species that emerges in early spring and nests in hollow twigs and beetle burrows (Bosch and Kemp 2001). We focused on two geographically widespread ectoparasitoids commonly found in *O. lignaria* nests: *Monodontomerus torchioi* Grissell (Hymenoptera: Torymidae) and *Melittobia acasta* (Walker) (Hymenoptera: Eulophidae). We selected these species because they differ in both their oviposition strategy and the breadth of their host range. To parasitize the host, *Monodontomerus* females oviposit directly through the host cocoon onto the prepupa or pupa (Eves 1970, Bosch and Kemp 2001, Filella et al. 2011). In contrast, *Melittobia* parasitoids chew through nesting substrates and cocoons to feed on host hemolymph and/or oviposit (Hobbs and Krunic 1971, Cusumano et al. 2010). Unlike *Monodontomerus, Melittobia* spp. attack a wide diversity of insects, including Coleoptera, Diptera, and Hymenoptera (Hobbs and Krunic 1971, González et al. 2004, Silva-Torres et al. 2005, Cusumano et al. 2010).

Using *M. torchioi* and *M. acasta* reared from field-collected *O. lignaria*, we tested attraction to several host volatiles. Given that host bees are enclosed in a thick cocoon covered in layers of frass during the preferred life stages for parasitoid oviposition (prepupae, pupae), we expected both parasitoid species to be more attracted to volatiles associated with host frass and cocoons than volatiles emitted by the adult host. Our primary goals were (1) to determine which host-associated volatiles the two species use during host location and (2) to experimentally test parasitoid attraction to two previously-identified *Osmia* nest volatiles, acetic acid and methanol (Filella et al. 2011).

Materials and methods

Parasitoid collection and rearing

In February 2014, we attached seventy-five pine nests (15 × 14 × 15 cm) containing paper nest tubes to narrowleaf cottonwood trees (*Populus angustifolia*) on a 7 ha. tract of land in Mountain Green, Utah (41°10'2"N 111°41'35"W). In July 2014, we collected all nesting tubes and transferred them to an incubator for eight weeks (28°C) to allow occupants to complete development. Using benchtop digital x-ray analysis (8 sec exposure at 20 kVp), we identified and isolated parasitoids within the nests. The two most abundant parasitoids were *Monodontomerus torchioi* and *Melittobia acasta*. We stored parasitoids at overwintering conditions (3–4°C) from October 2014–March 2015. In March 2015, we transferred the larvae to an incubator (31°C, 60% RH) and reared them to adulthood in ventilated plastic soufflé cups (Dart Corporation, Lodi CA). Upon emergence, we transferred all adult parasitoids to a second incubator (21°C, 60% RH). We fed *M. torchioi* adults a 50% honey solution *ad libitum*. We did not provide honey solution to emerged *M. acasta* because this species feeds directly on host hemolymph (Silva-Torres et al. 2005). We isolated virgin females with three males in soufflé cups 24 hours prior to testing and tested them in a Y-tube bioassay 2–3 days after emergence.

Testing

We used a Y-tube bioassay to test parasitoid attraction to four host volatiles: *O. lignaria* frass (0.01 g), live female *O. lignaria* adult in cocoon with frass removed, pure methanol (0.5 ml), and pure glacial acetic acid (0.1 ml). Previous research has identified methanol and acetic acid as the primary chemical components of *Osmia* cocoons and frass, respectively (Filella et al. 2011). We applied the methanol and acetic acid treatments to filter paper and allowed it to dry in the fume hood for at least 10 minutes prior to testing. We tested all treatments against blank filter paper.

The Y-tube olfactometer consisted of an air source pumped over an activated charcoal filter (5×100 cc/min) connected to an air flow monitor and divided into two substreams using Nalgene tubing (Fig. 1; Takabayashi and Dicke 1992). Tubing was connected to two glass bell jars (47 cm height × 15 cm diameter), one containing an odor source and the other containing blank filter paper (control). Nalgene tubing connected the bell jars to a glass Y-tube (Y-tube arms 1.2 cm diameter, 6 cm in length). To create an airtight seal, we positioned each jar in a plastic well filled with 1-2 cm of distilled water.

Using a clean strip of filter paper, we introduced a single naïve female parasitoid into the vestibule of the Y-tube. We recorded a "choice" if the female traveled at least halfway down a Y-tube arm. If the female did not make a choice within 5 minutes, the trial was recorded as "no choice." We tested a total of 82 *M. torchioi* females (n=20–21

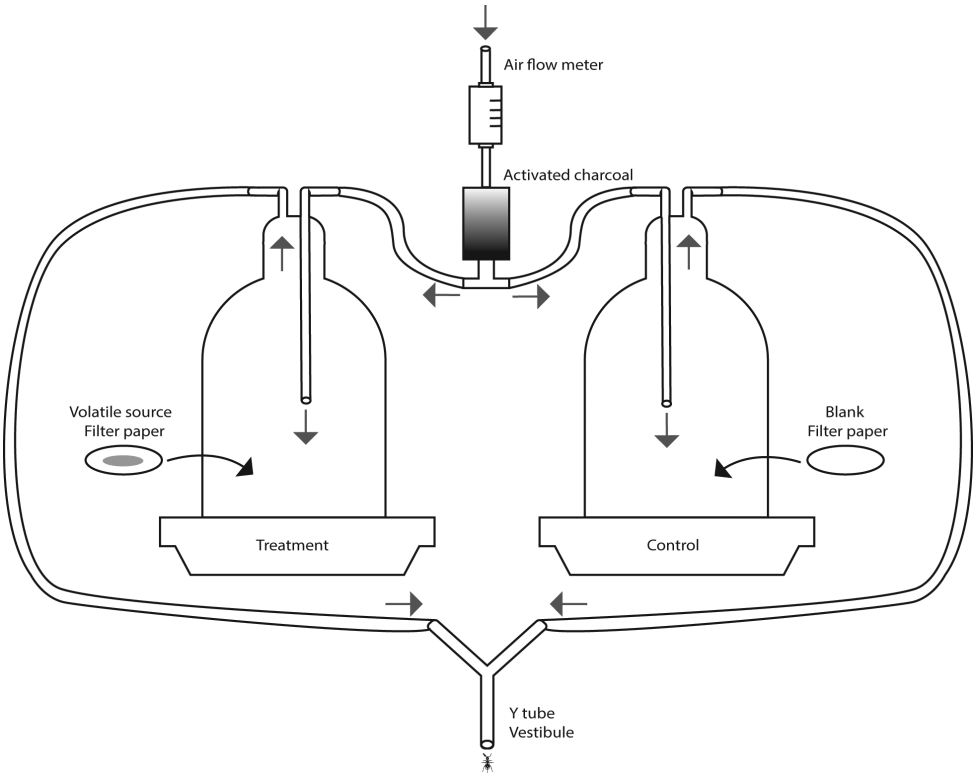

Figure 1. Schematic of the Y-tube olfactometer used to test parasitoid attraction to host volatiles.

per treatment) and 122 *M. acasta* females (n=30–32 per treatment). Although we initially planned to test the same number of individuals per treatment, two *M. torchioi* and six *M. acasta* escaped from containment prior to testing.

In an effort to maintain consistent environmental conditions, we placed visual blocks around the perimeter of the testing arena and used overhead full spectrum fluorescent lights to simulate natural light. In addition, ambient temperature within the testing arena remained at a constant 22°C throughout the study. After each trial, we washed glassware in detergent and rinsed it with distilled water and pure ethanol. To ensure volatile residue did not interfere with testing, tubing and glass bell jars were associated with a single treatment during testing. Lastly, we switched the position of the treatment and control bell jars following each trial to reduce directionality bias.

Data analysis

We tested whether the number of individuals that selected treatment over the control differed significantly from 1:1 using exact binomial tests, assuming a 50% chance of choosing either arm of the Y-tube (R Core Development Team 2016). Tests compared

the number of parasitoids that chose (1) *Osmia lignaria* frass or control, (2) live cocooned *Osmia lignaria* female or control, (3) methanol or control, and (4) acetic acid or control. Individuals that did not make a choice were excluded from the analysis.

Results and discussion

Across all trials, 70–90% of *M. torchioi* and 73–93% of *M. acasta* made a choice. Both species showed strong attraction to several of the volatiles tested, suggesting that they respond to multiple olfactory cues when searching for a host. Significantly more *M. torchioi* and *M. acasta* females were attracted to host frass and its primary chemical component, acetic acid, than the control (Table 1). These results empirically confirm previous work suggesting these volatiles act as chemical cues for parasitoid females (Agelopoulos et al. 1995, Sullivan et al. 2000, Filella et al. 2011). Specifically, frass and its associated volatile components may indicate host suitability. In general, *Monodontomerus* and *Melittobia* parasitize host pre-pupae and pupae (Eves 1970, Dahms 1984). In this system, *O. lignaria* defecate as fifth instar larvae, later incorporating this frass into the cocoon with salivary strands (Torchio 1989). Thus, frass-associated volatiles may allow parasitoids to discriminate between host life stages.

In contrast, the parasitoid species showed substantially different reactions to methanol. We expected both species to be attracted to methanol because it is a primary chemical component of host cocoons (Filella et al. 2011) and a natural byproduct of wood rot (Arantes and Goodell 2014). Megachilid bees often nest in standing dead trees (Macivor and Salehi 2014), therefore we hypothesized that methanol may act as a general cue that allows parasitoids to locate host nests. Although a higher number of *M. torchioi* females selected methanol over the control, this difference was not statistically significant (Table 1a). Using exact binomial testing with a larger sample size would be needed to assess the significance of these trends. Contrary to our expectations, *M. acasta* appeared to be repelled by methanol (Table 1b). It is possible *M. acasta* females avoided the methanol treatment because the concentration was too high. An alternative explanation for this behavior is that methanol acts as a repellent when isolated from other host-associated volatiles. The ratio-specific odor recognition hypothesis presented in Bruce et al. (2005) stresses that correct ratios and combinations of common plant volatiles are more generally used by phytophagous insects than any one species-specific volatile source. Furthermore, Takemoto and Takabayashi (2015) observed repellent effects of an isolated compound, linalool, regardless of concentration. However, the parasitoid was attracted to linalool when in combination with other herbivore-induced plant volatile components. Additional studies that test attraction to multiple methanol concentrations and blends of host-associated volatiles are necessary to test this hypothesis.

Lastly, *M. acasta* was strongly attracted to cocooned *O. lignaria* females, but *M. torchioi* was not (Table 1). Our results for *M. acasta* are consistent with previous research showing *Melittobia* rely primarily on indirect host-associated chemical cues,

Table 1. Results of choice tests for *Monodontomerus torchioi* (Hymenoptera: Torymidae) (**A**) and *Melittobia acasta* (Hymenoptera: Eulophidae) (**B**) Mated females of both species were tested for attraction to four host-associated volatiles: acetic acid, frass, methanol, and cocooned *O. lignaria* adult female.

A.

	No. individuals that chose			
Volatile source (n)	**Treatment**	**Blank**	**Neither**	**P**
Acetic acid (20)	13	4	3	0.049*
Frass (21)	14	4	2	0.031*
Methanol (20)	11	3	6	0.057
Cocooned *O. lignaria* female (21)	11	8	2	NS

B.

	No. individuals that chose			
Volatile source (n)	**Treatment**	**Blank**	**Neither**	**P**
Acetic acid (32)	22	7	3	0.008**
Frass (30)	26	2	2	<0.0001***
Methanol (30)	4	20	6	0.002**
Cocooned *O. lignaria* female (30)	20	2	8	<0.0001***

rather than cues produced by the appropriate host stage (Cusumano et al. 2010). Because parasitoids in this group employ a 'sit-and-wait' strategy in the host nest (Cusumano et al. 2010) and are limited in their dispersal ability (Matthews 2009), a strong attraction to the adult host may also allow them to locate nests at short distances. Surprisingly, *M. torchioi* was not attracted to volatiles from cocooned host bees (Table 1). Given that the closely-related *M. aeneus* (Fonscolombe) does not respond to volatiles from uncocooned *Osmia* host prepupae but is attracted to host cocoons and frass (Filella et al. 2011), it is possible that *Monodontomerus* spp. rely on a combination of volatiles emitted by the host at the preferred life stage. To disentangle parasitoid response to adult hosts and cocoons, future studies could examine attraction to uncocooned *Osmia* adults.

Our study is among the first of its kind to test parasitoid attraction to pollinator-associated volatiles. Collectively, our results suggest that both *M. torchioi* and *M. acasta* use a number of volatile cues to locate hosts. By simultaneously comparing parasitoid attraction to multiple volatiles and combinations of volatiles, future studies may allow us to rank the relative importance of each cue and further elucidate the complexities of parasitoid host location.

Contribution of authors

SG contributed to data collection and writing. SF contributed to data collection and analysis, writing, and project design.

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