JOURNAL OF THE KANSAS ENTOMOLOGICAL SOCIETY
92(4), 2019, pp. 649-654

SHORT COMMUNICATION

Clear Plastic Bags of Bark Mulch Trap and Kill Female *Megachile* (Hymenoptera: Megachilidae) Searching for Nesting Sites

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ABSTRACT: In 2017, we found 17 dead females of *Megachile frigida* Smith in clear plastic bags of composted bark mulch in a residential yard in Bozeman, Montana, USA. Females apparently entered bags via small ventilation holes, then became trapped and died. To investigate whether this is a common source of mortality, we deployed unmodified bags of mulch and those fitted with cardboard tubes (as potential nest sites) at three nearby sites in 2018. We found two dead *M. frigida* females and five completed leaf cells in one of these bags of mulch fitted with cardboard tubes; two male *M. frigida* emerged from these leaf cells. In 2018, we also discovered three dead female *M. frigida* and three dead females of a second leafcutter bee species, *Megachile gemula* Cresson, in clear bags of another type of bark mulch. Both mulches emitted nearly identical blends of volatile organic compounds, suggesting their odors could attract females searching for nesting sites. These findings suggest that more research is needed to determine how common and widespread this is for *Megachile* species that nest in rotting wood and if there are simple solutions to this problem.

KEYWORDS: Leafcutter bees, solitary bees, cavity-nesting bees, Apoidea, wild bees, pollinators, *Megachile frigida, Megachile gemula*

The leafcutter bees *Megachile frigida* Smith, 1853 and *Megachile gemula* Cresson, 1878 (Megachilidae) are widespread in North America (Mitchell 1960; Michener, 2007; Sheffield *et al*., 2011). Both belong to the subgenus *Xanthosarus* Robertson and are solitary, cavity-nesters whose females individually provision nests for their offspring (Michener, 2007). *Megachile frigida* have been recorded nesting in rotting logs and other dead wood (Hobbs and Lilly, 1954; Pengelly, 1955; Stephen, 1956), as well as in trap-nests made from wood (Jenkins and Matthews, 2004). Less is known about the nesting habits of *M. gemula*, though they have been observed nesting in trap-nests made from bored plant stems, including elderberry (*Sambucus*) and sumac (*Rhus*), and using fine wood chips to plug completed nests (Fye, 1965; Medler and Lussenhop, 1968). Here, we report observations that females of both species are attracted to and enter bags of bark mulch, where they can become trapped and die. We also characterized the volatile chemicals emitted by two mulches that could play a role in attracting bees.

Our initial observation occurred on 23 July 2017 in Bozeman, Montana where we noticed a female leafcutter bee flying around the outside of several unopened, clear, plastic, 0.06-m^3

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Received 16 October 2019; Accepted 18 May 2020

(2-ft^3) bags of composted mulch marketed as "Soil Pep®" (Mountain West Products, Rexburg, ID), that had laid on the ground in the sun for approximately two weeks. Soil Pep® is commonly used as a soil amendment or top dressing in landscaping (http://www.mountainwestbark.com/products/38-minus-composted-soil-pep/) and, according to the label on the bags, is composed of "screened fir and pine bark fines". The bee flew off, but several minutes later, we heard buzzing sounds of a bee trapped inside the bag beneath the clear plastic. When we opened the bag, the bee dug deeper into the mulch. When retrieved, the bee had stopped moving and her tongue was extended, though she recovered after being placed in nearby shade. At the time, and during the next several days, we noticed additional, medium-sized (13-15 mm) female *Megachile* actively investigating the opened bags of mulch. Upon further examination of four bags of Soil Pep®, we found a total of 17 dead females (and no males) in two of them. All dead bees were identified as *M. frigida* using keys in Sheffield *et al.* (2011), and comparing them to specimens in the O'Neill Laboratory, Montana State University. The majority of dead bees (at least 11) were found just beneath the plastic on top of the mulch. Perhaps they had started from the ventilation holes on the sides of the bags and dug their way to the top of the mulch where they could not escape. There is also the possibility that some of the dead adult *Megachile* originated from offspring of females that were already nesting in the mulch prior to packaging so that the bees in the bags were their offspring. However, it seems unlikely they would have tolerated the thermal conditions in bags during the final stages of their development. Additionally, we likely would have seen at least one male among the dead bees assuming the typical strongly male-biased sex ratio among *Megachile* broods (e.g., Krombein, 1967; O'Neill and O'Neill, 2010). Finally, as noted below, we were able to induce *M. frigida* females to nest in the bags at our site by providing non-collapsible cavities for nesting within the bags.

These observations led us to explore whether bags of Soil Pep® might be a common source of bee mortality for *M. frigida* or other species of *Megachile* with similar nesting habits. Bags of Soil Pep® and similar types of mulch are often kept stacked in yards or on pallets in parking lots at garden centers and home improvement stores where bees could readily access them (pers. obs.). Several cues might make the bags attractive to bees. Females might be attracted to small, 6-mm diameter, circular holes in the bags that allow airflow and reduce moisture build up (Fig. 1A). In addition, the clear bags of dark brown mulch resemble logs. Perhaps in addition to visual cues, females might be attracted to volatiles emitted by the mulch if those chemicals are similar to those emanating from rotten wood of the type where they naturally nest. After the bees enter bags and begin tunneling, it is likely that the finely-textured mulch would collapse on them, making it difficult for them to retrace their path to the entrance hole. This could be exacerbated by the fact that the bags are clear, therefore they cannot use any light cues to guide their way specifically in the direction of the ventilation holes.

On 9 July 2018, we deployed one each of two types of bags of Soil Pep® (the same type in which dead bees were found in 2017) at three locations in Gallatin Co., Montana (Site 1: 45.678°N, 111.048°W; Site 2: 45.667°N, 111.071°W; Site 3: 45.644°N, 111.1585°W). The first type was simply unmodified bags of Soil Pep®. The second type was Soil Pep® bags with cardboard tubes (6-mm internal diameter, 15-cm length; Custom Paper Tubes, Valley View, OH) inserted into all the ventilation holes (Fig. 1B). These tubes are readily adopted as nests by cavity-nesting bees and wasps (e.g., O'Neill and O'Neill, 2010), though we have never recorded *M. frigida* using these tubes in any of our surveys of trap-nesting bees in Montana (pers. obs.). We hypothesized that the cardboard tubes would add structural integrity to their tunnels, much like a rotting log might have a matrix of sound and partially rotted wood, reducing the probability that bees would

Figure 1. Bags of Soil Pep®. (**A**) unmodified bag showing 6-mm diameter, circular ventilation holes; (**B**) modified bag with cardboard tubes (6-mm internal diameter) inserted into ventilation holes.

become trapped in the bag (as long as they remained in the tube) and increasing the probability they could provision cells that produced offspring. Each of two bag types was used at three sites around Bozeman in unshaded locations and left out until late August.

On 25 August, we opened all bags of Soil Pep® and sieved all contents to look for dead bees and/or nesting activity. In total, we found two dead females of *M. frigida* in a Soil Pep® bag fitted with cardboard tubes (at Site 2). As in 2017, the bees were found just beneath the plastic on top of the mulch in the unopened bag. We also found five completed leaf cells within the cardboard tubes in the same bag; some were partially within the cardboard tubes whereas others were loose in the Soil Pep®. It was not clear if these cells were all part of one nest created by an individual female or multiple nests created by one or multiple females. These leaf cells were placed in cold storage at 6 °C on 5 November 2018. On 20 May 2019 the leaf cells were removed from cold storage and placed at room temperature to initiate emergence of bees; on 21 June 2019, two male *M. frigida* emerged. We dissected the remaining three leaf cells and found one cell without a cocoon, but with exuviae of dermestid (Dermestidae: Coleoptera) larvae; two others contained *Megachile* cocoons with dead prepupae.

In 2018, we observed another instance of bee mortality in mulch. At Site 1, another type

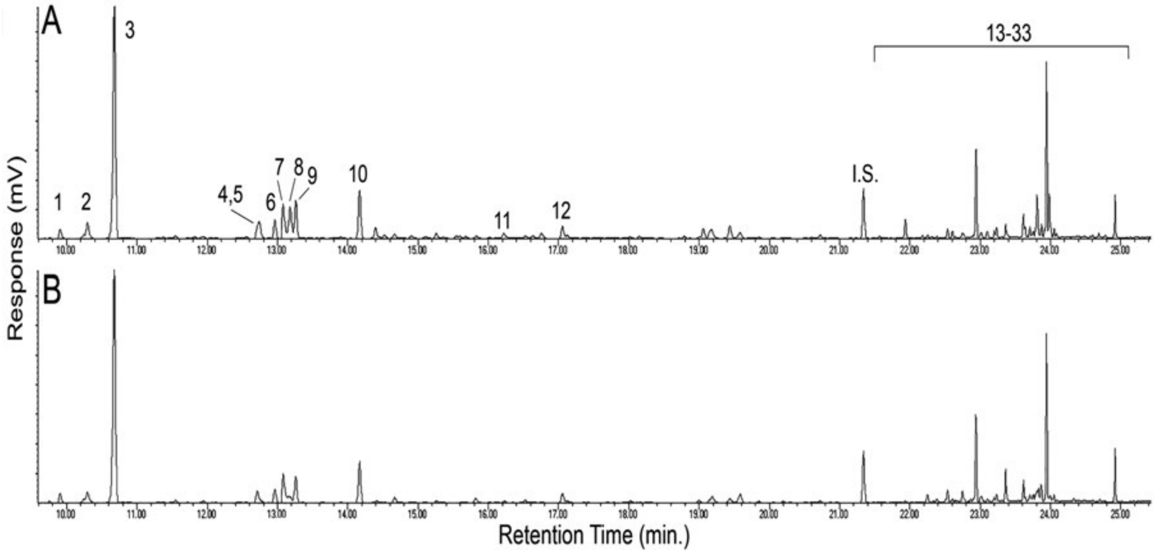

Figure 2. Total ion chromatograms showing VOCs collected for 30 min (1 L/min) from 50 g of **(A)** pine bark mulch and **(B)** Soil Pep®. Numbers correspond to compound identity of larger peaks: 1, tricylcene; 2, alpha-pinene; 3, camphene; 4, 3-carene; 5, unidentified monoterpenoid; 6, p-cymene; 7, unidentified monoterpenoid; 8, eucalyptol (1,8-cineole); 9, limonene; 10, gamma-terpinene; 11, camphor; 12, ethyl benzoate; 13-33, unidentified sesquiterpenes. I.S., internal standard.

of pine bark mulch (three unopened 0.06-m^3 bags of Greensmix®, Waupaca, WI) was present that was not part of the study. On 29 July, we found three dead females each of *M. frigida* and *M. gemula* in two of the three bags; the bags were clear plastic and had ventilation holes similar in size to those in the Soil Pep® bags.

The strong odor emitted from the mulches and the known natural nesting habits of *M. frigida* prompted us to collect and analyze the volatile organic compounds (VOCs) from the two mulches to identify chemical cues that might have attracted the bees (following methods in Runyon and Birdsall, 2016). VOCs were collected by enclosing 50 g of mulch (N = 3 each of Soil Pep® and pine bark mulch, each sample from a different bag) in 2 L glass domes and pulling air for 30 min at 0.5 L/min through a VOC trap containing 30 mg of the adsorbent HayeSep-Q (Restek, Bellefonte, PA) using portable volatile collecting systems (Volatile Assay Systems, Rensselaer, NY). VOCs were eluted from traps with 150 µl of dichloromethane, and analyzed using an Agilent 7890A gas chromatograph (GC) coupled with a 5975C mass spectrometer and separated on a HP-1ms column (30 m × 0.25 mm inside diameter, 0.25 µm film thickness); helium was used as the carrier gas. The GC oven was maintained at 35 °C for 3 min and then increased by 5 °C per min to 125 °C, then 25 °C per min to 250 °C. Identifications of compounds were made by comparing mass spectra and retention times with commercial standards.

The VOCs emitted by pine bark mulch and Soil Pep® were very similar to one another and dominated by terpenes (Fig. 2A-B). The same compounds were emitted by both mulches and the most abundant VOC was camphene. Ethanol was also detected in all samples (not shown in Fig. 2). Terpenes and ethanol are emitted from decomposing wood and can serve as host location cues for insects that feed on dead or dying trees (Chénier and Philogene, 1989). Females of

Megachile could be using VOCs from mulch bags to guide their nest searching behaviors. Some species of *Megachile* are known to use volatile cues from old nests when searching for nesting sites (e.g., odors from conspecific cocoons, fecal material on the outside of a cocoon, leaf pieces used to line nest cells; Pitts-Singer, 2007; Stanley and Pitts-Singer, 2011). We cannot rule out visual cues as the pine bark mulch was also in clear bags and might resemble a log to the bees. It seems likely that both visual and olfactory cues may be responsible for attracting females. Many species of solitary bees use cavities in wood as nest sites (e.g., Krombein, 1967; O'Neill and O'Neill, 2010), including species of *Megachile* in the Bozeman area (Jensen, 2001; unpublished data). However, although other species might have been attracted to the holes in the plastic bags, it is telling that most of the dead bees that we found were of a species known to burrow in dead wood. Thus, the presence of the mulch, as well as the holes, seems to be what induced bees to enter the bags and become trapped. Clearly, further research is needed to elucidate the specific array of cues used by *Megachile* when they are attracted to natural nesting sites or to mulches.

Trap-nests are the most common means of providing artificial nest sites for solitary bees (e.g., Krombein, 1967). However, other nesting substrates have been tried (e.g., overturned terracotta saucers, Sheffield, 2015; colloidal clay, Graham *et al.*, 2015; blocks of local soil, Fortel *et al.*, 2016). One of our bags of Soil Pep® fitted with cardboard tubes did allow for successful *M. frigida* nesting. However, we also found dead *M. frigida* females within this bag, suggesting that once bees exit the end of the cardboard tubes into the mulch they are subject to the same fate as those that enter bags without cardboard tubes. If bees are attracted to the odor of bark mulch when searching for nesting sites, another potential option for creating artificial nesting sites would be baiting blocks of trap-nests with quantities of Soil Pep® (e.g., packed into the back ends of nest tunnels), or perhaps even with individual or mixes of volatile chemicals identified from the Soil Pep® and pine bark mulch. However, further work on developing artificial nests, including the use of rotting wood, and exploiting chemical cues to attract and retain bees at nesting sites is needed.

In conclusion, we documented a behavior of *M. frigida* and *M. gemula* females that had fatal consequences at two locations in Bozeman, Montana and across multiple years, indicating that the 2017 observations were not an isolated event. However, we still do not know how widespread this phenomenon is for either of these two species, or other species that nest in rotting logs such as *Megachile inermis* Provancher and *Megachile pugnata* Say (Sheffield *et al.*, 2011). Our observations leave many questions unanswered, but we hope they prompt others to be on the lookout for this behavior in other parts of North America. If such mortality is common, further study may suggest simple solutions, such as modification of the types of bags used (e.g., color of the plastic, size of ventilation hole) and where and how the bags are stored (e.g., inside buildings or outside under tarps to reduce access by the bees).

ACKNOWLEDGMENTS

We thank James Cane for helpful discussions regarding this observation, Joshua Botti-Anderson for help with field work, and Michael Ivie for providing helpful comments on the manuscript. This project was supported by the Montana Agricultural Experiment Station and by the Montana Department of Agriculture's Specialty Crop Block Grant Program under the (Wild) Bees of Montana, SCBFP #18SC007084, M. A. Ivie, PI.

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