NEST, SOIL AND POLLEN PREFERENCES OF MASON BEES (HYMENOPTERA: MEGACHILIDAE: Osmia) IN SOUTHWEST MICHIGAN FARMS

By

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ABSTRACT

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Bees (Hymenoptera: Apoidea) are the most important pollinators due to their active collection of pollen and frequent visits to flowers. Although honey bees (Apis mellifera) are still the most economically important commercial crop pollinators, other bee species are being developed, including the mason bees (Hymenoptera: Megachilidae: Osmia), which are efficient pollinators of tree-fruit crops. However, we still need to improve the management of these bees and understand how they use resources in their environment. I studied the nesting preferences, soil use and pollen preferences of two common mason bees in Michigan: Osmia lignaria and O. cornifrons. Several nesting shelters and nesting substrates were offered to bees, revealing that the combination of wooden shelters with natural reeds and an attractant resulted in the most nesting. Analysis of soil brought back to the nest showed that most nest partitions are loam or sandy loam, with the two species showing different soil preferences. In the brood provision, even when the nests were in the center of a large blueberry field, O. lignaria did not use this crop as a dominant pollen resource. Results presented here will help to improve management of these bees by offering more suitable nesting materials and shelters, as well as highlighting that O. lignaria is likely not very suitable for blueberry pollination. The soil texture analysis provides new insights into the soil preferences and requirements of mason bees.

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CHAPTER 1. LITERATURE REVIEW: NESTING, POLLEN AND SOIL PREFERENCES OF MASON BEES (HYMENOPTERA: MEGACHILIDAE: *Osmia*)

INTRODUCTION

Seventy-five percent of crops around the world depend, to some extent, on animal pollination, and it is required by many crops to achieve economic yields (Klein et al. 2007). Bees (Hymenoptera: Apoidea) are the most important pollinators due to their active collection of pollen and frequent visits between conspecific flowers (Gallai et al. 2009, Garibaldi et al. 2011, Nogué et al. 2016), contributing approximately \$14.6 billion per year in the United States, of which \$3.07 billion is provided by wild bees (Morse and Calderone 2003, Losey and Vaughn 2006, Klein et al. 2007). European honey bees (*Apis mellifera* L.) are the most commonly used commercial pollinator, however, in the last few decades their populations have declined (VanEngelsdorp et al. 2008, Jaffé et al. 2010, Potts et al. 2010, VanEngelsdorp and Meixner 2010). Decreasing honey bee populations have sparked an interest in studying alternative pollinators, which can be more efficient per visit than honey bees for many crops (Garibaldi et al. 2013).

Bees in the genus *Osmia* (Megachilidae) are an important group of alternative pollinators. These solitary, cavity-nesting bees emerge early in the spring, and some species have a strong preference for tree-fruit crops, which makes them good pollinators of these crops (Bosch and Kemp 2001, Mader et al. 2010). In the United States, *Osmia lignaria* Say and *O. cornifrons* (Radoszkowski) are the two species with the highest potential to be used in commercial orchards (Mader et al. 2010, Sedivy and Dorn 2014). Several strategies have been developed to rear and release *Osmia* in crops, but there are still issues related to how to improve their retention and

reproductive success in commercial orchards (Biddinger et al. 2008, Sedivy and Dorn 2014). Variables such as the number and location of nesting sites, number of bee release sites, type of nesting materials, effect of landscape structure and use of chemical nesting attractants still need to be assessed (Artz et al. 2013, 2014). Furthermore, the few studies that have considered *Osmia* species as potential pollinators of non-Rosaceae plants such as blueberry (*Vaccinium* spp.), found that some species can be effective depositing pollen on the stigma (Stubbs et al. 1997, Dogterom 1999, West and McCutcheon 2009, Bushmann and Drummond 2015), so the potential of *Osmia* bees as pollinators of other crops should be assessed in the field.

Alternative pollinators

There are over 20,000 described bee species worldwide (Michener 2007), and the majority collect pollen as the primary source of protein for their larvae. In many cases, wild bees can be better pollinators than honey bees due to specialized behavioral and morphological adaptations to specific flowers (Garibaldi et al. 2015). Although honey bee hives are commonly installed in many crops during bloom to ensure pollination, wild bees can greatly contribute to pollination (Garibaldi et al. 2013) and in some cases they can be the dominant pollinators, providing enough pollination for small crop fields and making supplementary pollination unnecessary (Ratti et al. 2008, Winfree et al. 2008, Isaacs and Kirk 2010). Furthermore, pollinator richness can increase yield and reduce the variance in yield between fields and across years due to complementary pollination among species (Morris et al. 2010, Blitzer et al. 2016, Garibaldi et al. 2016). Mason bees within the genus *Osmia* are one potential source of alternative pollinators.

Life cycle of mason bees

Osmia spp. are univoltine (one generation per year), solitary, cavity-nesting bees (Levin 1966), present mainly in the norther hemisphere (Michener 2007). In Michigan, adults emerge early in the spring, around April and May, with males emerging approximately 3-6 days earlier than female. Upon emergence, females mate and start locating nesting locations (Torchio 1989, 1990) (Fig 1.1). Usually, mason bee populations are biased towards males, with 1.5 - 2 males per females (Torchio and Tepedino 1980). In the wild, mason bees nest mainly in the soil or in existing cavities made by wood-boring insects, but they can also nest in fabricated structures such as wood blocks with drilled holes, cardboard straws, hollow reeds or bamboo (Levin 1966, Bosch and Kemp 2002, Cane et al. 2007, Mader et al. 2010).

Females of *O. lignaria* and *O. cornifrons* tend to return to the locations where they emerged, attracted by volatiles present in old cocoons (Torchio 1984, Pitts-Singer 2007). Once they select a nesting cavity, it is marked with free fatty acids, hydrocarbons and wax esters that allow bees to identify the cavity and warn other females that it is in use (Raw 1992, Guedot et al. 2006). This behavior is especially useful for species that tend to nest in close proximity to other individuals, allowing them to distinguish individual nests (Fauria and Campan 1998).

Nests of *O. lignaria* and *O. cornifrons* usually consist of straight cavities in wooden substrates. Females clean the selected cavity and build a partition at the back of the nest (Torchio 1989). Some species use mud to make the partitions between cells (Bosch and Kemp 2000), while other species in the genus use leaves or a mix of plant materials and mud when partitioning cells (Torchio 1989, Cane et al. 2007, Sampson et al. 2009). Once the first partition is built, females start foraging for pollen and nectar, with 15 to 40 foraging trips required to fill one brood cell (Levin 1966, Torchio 1989, Mader et al. 2010). Although some species can fly as far a 1200 m (Guedot et al. 2009), their effective foraging range is estimated to be between 100-200 m (Bosch and Kemp 1999), and most of the foraging activity occurs within 30 to 50 m of the nest when abundant resources are available (Monzón et al. 2004, Matsumoto et al. 2009, Biddinger et al. 2013).

Females then lay a single egg directly on the pollen ball and start collecting soil to build a partition (Torchio 1989, Bosch and Kemp 2000). Four to 12 cells are typically built in each cavity, with the highest probability of female eggs in the innermost cells, and male eggs in the cells closest to the entrance of the nest. With good conditions females can build between 1 to 6 nests within one month (Medler 1967, Torchio 1982a, 1984, Rust 1998).

Osmia spp. larval development is temperature dependent (Bosch and Kemp 2003, Kemp and Bosch 2005), and for *O. lignaria*, development from larva to pupa can take approximately 97 days in field conditions (Bosch and Kemp 2000). After reaching the final instar, larvae spin a cocoons and pupate in it (Torchio 1989) (Fig 1.1), with female cocoons being larger than male cocoons (Torchio and Tepedino 1980). Bees finish their development by August, after which they are required to go through a winter diapause to successfully emerge the next spring. If bees are deprived of this cold period, they will consume their fat tissues and starve (Sgolastra et al. 2011, 2012). Overwintering adults will remain in their cocoons until next spring, with temperature increase triggering emergence (White et al. 2009) (Fig 1.1).



Figure 1.1. *Osmia* life cycle, presenting the main development stages of the bee. From Mader et al. (2010).

Mason bees (Osmia spp.) as crop pollinators

Mason bees are common pollinators of early spring plants, including crops such as apple, cherry, pear and plum (Bosch and Kemp 1999, Ladurner et al. 2004, Monzón et al. 2004, Matsumoto et al. 2009, Mader et al. 2010, Sheffield 2014). For this group of rosaceous plants, mason bees are considered more efficient pollinators than honey bees because they mainly forage for pollen, constantly make contact with the reproductive parts of the flowers, and can fly at lower temperatures than honey bees (Bosch and Kemp 2001, Mader et al. 2010, Matsumoto and Maejima 2010). Importantly, they tend to gather pollen from at least two cultivars during foraging trips, promoting cross pollination (Matsumoto et al. 2009).

Osmia species that have been studied as alternative commercial pollinators include *O. lignaria* in the USA (Bosch and Kemp 2000, 2002), *O. cornifrons* in Japan (Matsumoto and Maejima 2010) and *Osmia cornuta* (Latreille) in Europe (Bosch 1994a). In Japan, *O. cornifrons* has been used as an orchard pollinator since the 1940s, and was introduced to the United States in 1977 by the USDA Bee Lab in Beltsville, Maryland (Batra 1978). *Osmia lignaria* is native to the USA, with two subspecies; *O. lignaria propinqua* Cresson and *O. lignaria lignaria* Say, which are found in western and eastern regions of the continent, respectively (Rust 1974). In some areas, *Osmia* species are commercially available and their emergence time can be manipulated with temperature to coincide with bloom of different crops such as almond (Bosch and Kemp 2000, Artz et al. 2014) and blueberry (West and McCutcheon 2009).

Even though *Osmia* bees are recognized as good pollinators of tree crops, their populations in orchards can be at low levels (Vicens and Bosch 2000a, Gardner and Ascher 2006, Schlueter and Stewart 2015). If an effective pollinator is not present in adequate numbers, increasing the density of pollinators can help to achieve a high pollination rate. One example is the case of honey bees pollinating blueberries, in which the fruit-set increases when the number of visits per flower increases (Dedej and Delaplane 2003). In the case of stem nesting bees, the number of bees in the field can be increased by providing suitable nesting options and/or by releasing individuals in the field. Some studies report a substantial increase in *Osmia* populations in crops after releasing an initial group of females and providing adequate nesting materials (Bosch and Kemp 1999, Bosch et al. 2006, Krunic and Stanisavljevic 2006).

Given that mason bees need to go through a period of cold temperature to be able to emerge in the spring (Sgolastra et al. 2011), several studies have addressed the effects of temperature, pre-wintering and winter duration on mason bee development, survival and

emergence time (Bosch and Kemp 2003, 2004, Kemp and Bosch 2005, Sheffield, Westby, Kevan, et al. 2008, Radmacher 2011, Sgolastra et al. 2011, 2012). This knowledge allows for control of bee emergence to match with crop bloom. The recommended density for *O. lignaria* is 620 and 740 females/ha in apples and almond orchards, respectively. For apples, bees can be released when 30% of flowers are open (Vicens and Bosch, 2000a), while for almonds it is recommended to release bees when 5-10% of flowers are in bloom (Artz et al. 2013).

Recently, in order to improve the use of these bees in crops, the effect of pesticides on mason bees has been studied in more detail. Several authors report that *Osmia* species can be more sensitive than honey bees to some insecticides, and that fungicides, even without a lethal effect on bees, can disrupt the nest recognition abilities (Ladurner et al. 2005, 2008, Biddinger, Robertson, et al. 2013, Artz and Pitts-Singer 2015). Assessment of nesting substrates/shelters have been running for many years (Torchio and Tepedino 1980, Torchio 1982a, 1982b, 1984, Artz et al. 2014), but there are still many questions about their management that need to be answered to achieve sustainable and reliable use of these bees in agriculture.

Nesting preferences of Osmia

In order to develop effective strategies for managing mason bees as commercial pollinators, it is essential to understand their nesting preferences given that a lack of nesting sites can limit the size of wild populations (Yoon et al. 2015). Studies describing mason bee nesting date back to 1925 (Fabre 1925) with later reviews of their biology (Levin 1966, Raw 1972). Species such as *O. lignaria* and *O. cornifrons* also have a natural tendency for building their nests in close proximity to other conspecific bees (Pitts-Singer 2007). This gregarious behavior facilitates

maintaining a large number of nests and individuals in a single place that can be used for crop pollination (Mader et al. 2010).

The reported preferred nest dimensions for *O. lignaria* are 7.5 mm diameter holes, that are 152 mm deep (Mader et al. 2010). However, Torchio (1984) reports that dimensions seem to be less important than the attractiveness of the nesting material for this species. *Osmia cornifrons* prefer nests 5.8-6.0 mm in diameter, and 120-140 mm deep. Inter-hole distance is also important and is suggested to be 20 mm, facilitating orientation and nest location (Mader et al. 2010). The sex ratio usually varies between 1.5 to 2.0 males per female, and is partly determined by the depth and diameter of the nest cavity, with deeper tunnels favoring the production of females and narrower tunnels favoring males. Bees commonly build 5 to 13 cells per nest, with a decrease in the number of cells per nest later in the flight period. Nests built at this time also tend to contain a higher proportion of males (Torchio and Tepedino 1980, Mader et al. 2010). Female cells are bigger than male cells and contain larger pollen provisions, resulting in sexual dimorphism with females being bigger than males (Torchio and Tepedino 1980).

For management of large numbers of *Osmia* bees, nest shelter design should also be taken into account. Nests need protection from direct sunlight and rain with shelters, that can also serve as a visual landmark for bees (Mader et al. 2010). Shelters should not be moved after bees have started nesting; this will result in females abandoning their nests (Vicens and Bosch 2000c). Shelters that are more than 0.9 m depth are too dark and less attractive to bees (Maccagnani et al. 2007). Painting the shelters in colors that contrast with the landscape, or creating contrasting patterns as visual clues for bees can help females to easily identify their nest location (Fauria and Campan 1998). Torchio (1982a, 1984) recommends placement of the shelters facing east or

south to encourage flight activity early in the day. However, the direction of the nest does not affect the number of females nesting in a location (Yoon et al. 2015).

Dispersal away from the nest site is also a factor that can limit the effective use of mason bees as pollinators (Bosch and Kemp 2002). Osmia bicornis (L.) dispersal distance is very low with most bees dispersing less than 100 m (Gruber et al. 2011). Large females are less likely to exhibit pre-nesting dispersal (Steffan-Dewenter and Schiele 2004, Bosch and Vicens 2006), as well as bees forced to chew out of their natal nests (10-15% dispersal) (Bosch and Kemp 1999). If loose cocoons are used, they should be released in an emergence box consisting of a dark container with a single exit hole (Mader et al. 2010), but even in this case dispersal rates can be as high as 48% for O. cornuta (Bosch 1994), and can increase up to 81% for the same species when releasing emerged adults (Bosch 1994b). Bosse et al. (2014) report that performing multiple releases can increase the number of O. bicornis females nesting in a location, likely because females from the second release are attracted to the location where females from the first release are nesting because of their gregarious nesting behavior. Dispersal rates can also be reduced when bees are released in separate small groups, or by increasing attraction to nesting material (Bosch 1994a). Rough handling of pre-emergent adults does not affect the establishment of O. lignaria females when they emerge from their natal nest (Stanley et al. 2011), but the effect of rough handling on bee dispersal rate still needs to be assessed when managing loose cocoons.

Cavities that have been previously occupied by bees can be more attractive to nesting bees than new adjacent holes, however, the risk of diseases and parasites is higher in the old nests (Torchio 1984). Females may prefer to nest close to the place where they emerged because of olfactory cues from the old nests. Pitts-Singer (2007) found that *O. lignaria* females show a positive olfactory response to cocoons in a Y-tube bioassay, suggesting that the bees could use

them as an olfactory cue that attracts them to nest in a specific place. Cues used by bees to select potential nesting places include visual orientation cues, chemical cues and physical characteristics of the nesting material (Pitts-Singer 2007).

Enhanced bee retention and nesting distribution in commercial orchards are important factors when developing a management system for alternative bee pollinators. Variables such as the number and location of nesting sites, number of bee release sites, type of nesting materials, effect of landscape structure and use of chemical nesting attractants should be assessed (Artz et al. 2013, 2014). *Osmia lignaria* and *O. bicornis* prefer nest sites near orchard perimeters and along bee-ways (roads where honey bee hives are placed during bloom) (Matsumoto and Maejima 2010, Gruber et al. 2011, McKinney and Park 2012). The amount of fallow land surrounding apple orchards in Germany was the most important parameter for the establishment of *O. bicornis* in the orchards (Gruber et al. 2011), presumably because this provided pollen resources when the orchard was not in bloom.

As distance to the release point increases, the number of nests started by *O. bicornis* decreases (Gruber et al. 2011). Both *O. lignaria* (Artz et al. 2013) and *O. cornuta* (Bosch 1994a) increase the number of nests built when more small shelters with fewer stems are used instead of few large shelters with many stems. This indicates that it is easier for bees to find shelters that are in high density (Artz et al. 2013).

Once *O. lignaria* females have already established their nest, they show little hesitation when entering the nest, and if they accidentally enter the wrong nest, they quickly exit the cavity and scan over to locate the correct one. This suggests that *O. lignaria* uses olfactory cues to recognize their nests (Guedot et al. 2006). When exiting the nest, females drag the tip of the abdomen along the entire tube, marking not only the entrance but the complete tube with free

fatty acids, long chain hydrocarbons and wax esters (Guedot et al. 2006). The amount of compounds that females deposit varies, and can also vary between the entrance and middle sections of the tube, indicating that the nest marking chemistry is unique for each female. As can be expected, females are sensitive to the replacement of the entrance and middle section of the nest, making them hesitate and increasing recognition attempts, or even abandoning the nest (Guedot et al. 2006). This behavior indicates that chemical labeling of the nest is essential for nest recognition and its role on bee retention should be studied in more detail.

Pollen used by Osmia

Bees collect pollen and nectar to feed their larvae. *Osmia lignaria* requires 15 to 40 foraging trips to gather the necessary pollen and nectar to provision one cell (Mader et al. 2010) and *O. cornifrons* females can provision a cell in about 30 minutes when weather conditions are good and pollen sources abundant (McKinney and Park 2012)

Osmia spp. show a strong preference for Rosaceae trees, and during the crop bloom period they mainly collect pollen from these plants (Mader et al. 2010). In a cherry and peach orchard in Utah, the percentage of crop pollen (*Prunus* spp.) in *O. lignaria* loads varied between 68 and 98% (Bosch et al. 2006). In apple orchards in Canada, *O. lignaria* pollen loads contained 72-93% apple pollen (Sheffield et al. 2008). Monzón et al. (2004) examined *O. cornuta* larvae fecal samples, and found that overall, 94.4% of the pollen was pear pollen, and 75.5% of the samples contained exclusively pear pollen. Vicens and Bosch (2000a) reported that 94% of the cells made by *O. cornuta* in apple orchards contain 100% apple pollen. This highlights the strong preferences they can have for tree-orchard crops if their abundance is high enough.

Mason bees also show strong floral preferences when they are in natural habitats.

Radmacher and Strohm (2010) found that even when there were 107 plant species blooming around the nesting site, *O. bicornis* collect almost exclusively oak and maple pollen, and 39% of the cell pollen consists of a single (>95%) pollen type. Felicioli et al. (2004) report that the pollen of anemophilous plants like *Salix* spp., *Acer* spp., and *Quercus* spp. can be very attractive for *O. cornuta* and *O. bicornis*.

Furthermore, some plants can be even more attractive than the actual crop for *Osmia* bees. In Virginia and North Carolina apple orchards, Kraemer et al. (2014) found that the most abundant pollen in *O. lignaria* cells was redbud (*Cercis canadensis*, Fabaceae), ranging from 45 to 82%, and only in the farm without redbud trees they found that the orchard pollen reach 53%. Other pollen types reported in *Osmia* spp. loads include pollen from the families Fabaceae (*Lupinus polyphyllus*) Asteracea, Ranunculaceae, Papaveraceae, and other genera like *Erica*, *Quercus, Brassica, Carya, Liquidambar* (Monzón et al., 2004; Radmacher and Strohm, 2010; Sheffield et al., 2008). The fact that some plants can be more attractive that the actual crop for *Osmia* bees make important to consider the alternative floral resources present in the crop area, given that floral resources planted aiming to support bees could distract them from visiting the crop of interest

Vaccinium pollination by Osmia

Blueberries (*Vaccinium* spp.) are an economically important crop that requires insect pollination. The flowers have poricidal anthers that release pollen by mechanical stimulus (McGregor 1976, Abrol 2015). Bumblebees are able to "buzz-pollinate", in which they vibrate their thoracic muscles when visiting the flowers, so the pollen is released (Buchmann and Hurley 1978). Other

wild bees such *Habropoda laboriosa* (Fab) are recognized as good blueberry pollinators (Cane 1997, Sampson and Cane 2000), but to provide high abundance of bees, honey bee hives are placed close to the fields during bloom to ensure good pollination levels (Scherm et al. 2001, Dedej and Delaplane 2003). However, many other native bees visit blueberry flowers, and *Osmia* species can be observed visiting the flowers (Dogterom 1999, Sampson and Cane 2000, Isaacs et al. 2009, Cutler et al. 2015). Dogterom (1999) found *O. lignaria propinqua* in 55% of 80 blueberry sites in British Columbia, Canada, but with low abundance. Bushmann and Drummond (2015) report nine *Osmia* species visiting blueberry field in Maine, and Tuell et al. (2009) report 13 *Osmia* species visiting blueberry flowers in southwest Michigan.

Many authors report *Osmia* species as potential pollinators of blueberries. Stubbs and Drummond (1997) report that *Osmia atriventris* Cresson deposits more pollen on lowbush blueberry (*Vaccinium angustifolium*) flowers per visit than *Bombus impatiens* Cresson and *A. mellifera* in a cage experiment in Maine. In Poland, *Osmia bicornis* provide good pollination levels to *V. corymbosum* under isolation (Wilkaniee and Giejdasz, 2003), and *O. cornifrons* provide the same pollination level as bumblebees in a cage study with *V. corymbosum* in West Virginia (West and McCutcheon 2009). *Osmia ribifloris* Cockerell is another potential pollinator of blueberries, because their visits led to a high fruit set in a single visit experiment in a screenhouse with *V. ashei* for Tifblue and Climax cultivars. However, the same species does not pollinate the Premier cultivar efficiently, due to the slipper-like shape of the flowers. Despite these promising results, fruits pollinated by *O. ribifloris* contained fewer seeds than fruits pollinated by honey bees (Sampson and Cane 2000, Sampson et al. 2004, 2013). However, most of these experiments were conducted in controlled situations, where bees have blueberry flowers as their only pollen and nectar source, and may not be a good representation of what happens in open field settings. Another difficulty that *Osmia* can face when used for blueberry pollination is that *Vaccinium* spp. have a very small opening and a deep corolla, which can make it difficult for this non-buzz-pollinating bee to access the anthers and release the pollen. Cultivars with wider corollas and longer stigmas have greater probabilities of being pollinated by *Osmia* (Felicioli et al. 2004).

Osmia lignaria propinqua is one of the few *Osmia* species commercially available for crop pollination, and is primarily used for pollinating orchard crops. However, in *V. corymbosum* var. Bluecrop, *O. lignaria* deposit a similar number of pollen grains on the stigmas as honey bees, and eighty-six percent of *O. lignaria* females captured at the nest entrances had blueberry pollen on their body (Dogterom 1999). It is still unclear what percentage of the pollen carried by bees belonged to blueberry. On the other hand, Sampson et al. (2009) do not recommend *O. lignaria* as a pollinator of blueberry because even if *O. lignaria* can produce viable progeny when confined on blueberry, this bee has a strong preference for tree crops.

In some regions nesting activity of natural population of *O. lignaria* occurs between early May to mid-June, coinciding with blueberry bloom (Dogterom 1999). In Michigan, *O. lignaria lignaria* fly between April and May, so their activity period does not completely coincide with blueberry bloom, which happens during May (Garcia-Salazar 2002). However, loose cocoons can be stored in cold temperatures safely for two months longer than the natural emergence period (Mader et al. 2010), and the emergence time can be altered to coincide with blueberry bloom. The efficiency of this bee for pollinating blueberries in the field remain to be tested in Michigan farms.

Soil use by Osmia

Most bees in the genus *Osmia* use leaf material or a mix of leaves and soil to build the partitions between cells (Cane et al. 2007); however, *O. lignaria* and *O. cornifrons* exclusively use soil as material for the partitions and nest plugs once the nest is complete (Torchio 1989, Mader et al. 2010). Therefore, soil is an essential resource for these bees (Krunic and Stanisavljevic 2006), yet there is relatively little known about the composition of the soil that they collect or the ecology of their soil collection.

Osmia lignaria females congregate at soil collecting sites, where the soil is wet and they have been reported to fly up to 54 m away from the nesting location to collect soil particles (Torchio 1989). Sometimes females can excavate tunnels in inclined soil surfaces to reach a soil layer of a particular moisture content, and several individuals can use the same excavated hole (Torchio 1989). An *O. lignaria* female gathers soil by scraping with her mandibles and forelegs, then shapes it to create a round pellet 2-3 mm in diameter using her forelegs, mandibles and the ventral surface of the posterior tip of the abdomen. Then she holds the pellet between her mandibles and flies back to the nest (Levin 1966). A similar behavior has been reported for *O. bicornis* (Raw, 1972 as *O. rufa*).

The average time spent collecting a soil pellet is about a minute (Levin 1966), and 8 to 12 soil collecting trips are necessary in order to build one cell partition (Torchio 1989). The soil is deposited as a foundation that circumscribes the inner wall of the tube. Additional soil is added until a solid wall is formed across the nest. More soil is then added to the outer perimeter of the partition which is then polished (Torchio 1989). Females of *O. californica* Cresson can spend on average 4 minutes depositing each soil ball into the nest, while *O. lignaria* females spend an average of 2 minutes (Levin 1966). The total time required by *O. cornifrons* to complete a

partition is 20 minutes (McKinney and Park 2012). When females finish the last cell, they build a soil plug at the nest entrance that for *O. lignaria* can be 4-8 mm thick. Usually the plug is protruding slightly from the nest entrance (Torchio 1989).

Few studies have addressed the soil preferences of mason bees and ground nesting bees, which is important because it is an indispensable resource for these bees. Cane (1991) studied the composition of soil used by ground-nesting bees for their subterranean nests, finding that the percentage of sand is one of the most important factors for nesting site selection for ground-nesting bees. Bosch (1994) tried to study *O. cornuta* soil preferences by offering soil of different textures in dispensers, but bees preferred to collect moist soil from the ground. There is still much to learn about mason bee soil preferences if we want to establish them as commercial pollinators in orchards, and questions related to soil selection plasticity, preferred soil texture, and variability among individuals, populations, and species regarding soil preferences still need to be addressed.

SUMMARY

Pollination is an essential input for many crops, and farmers of large commercial scale crops have depended on honey bees to ensure good levels of pollination. To reduce the pressure and dependence on honey bees, it is necessary to find alternative pollinators that can be managed and used in commercial orchards. Mason bees have the potential to be managed in tree-fruit crops, where they exhibit good performance and are considered better pollinators than honey bees. However, in order to achieve sustainable management of these bees, improve female retention, reproduction success and pollination delivery, we need to increase our understanding of several aspects of their nesting preferences, including the interaction between nest substrates and shelter

type, effect of shelter density, release location density, factors inducing a high dispersal rate of females, soil use and preferences, and explore whether they can be used in other non-Rosaceae fruit crops.

To address some of these questions, the objectives of this research thesis are to:

- 1) Elucidate the nesting material and shelters preferences of *O. lignaria* and *O. cornifrons* in and agricultural environment in Michigan.
- 2) Determine whether the attractant spray developed from *Osmia* cocoons is an effective way to increase nesting by *O. lignaria* and *O. cornifrons*.
- 3) Determine the effect of nest location on blueberry pollen collection by O. lignaria.
- 4) Characterize the texture of the soil used by O. lignaria in an agricultural environment.

CHAPTER 2. IMPROVING MASS NESTING OF TWO *Osmia* (HYMENOPTERA: MEGACHILIDAE) SPECIES BY NEST DESIGN AND AN ATTRACTANT SPRAY

INTRODUCTION

Many plants rely on animals to transfer pollen from anthers to the stigmas, and bees (Hymenoptera: Apoidea) are the most effective and common pollinators of angiosperm plants, including a large proportion of crops around the world (Klein et al. 2007, Gallai et al. 2009, Abrol 2015). The European honey bee (*Apis mellifera* L.) is the most commonly used commercial pollinator, however, in the last few decades their populations have declined in some parts of the world (VanEngelsdorp et al. 2008, Jaffé et al. 2010, Potts et al. 2010, VanEngelsdorp and Meixner 2010). This decrease in honey bee populations has sparked increased interest in studying alternative pollinators (Canto-Aguilar and Parra-Tabla 2000, Rader et al. 2009, Christmann and Aw-Hassan 2012, Holzschuh et al. 2012, Sedivy and Dorn 2014), with some studies showing that many other bee species are more efficient pollinators than honey bees in many crops, due to their behavioral and morphological adaptations to some kinds of flowers and their floral preferences (Javorek et al. 2002, Krug et al. 2010, Sheffield 2014, Junqueira and Augusto 2016).

Mason bees, *Osmia* (Megachilidae), are an important group of crop pollinators. Some species show high pollination efficiency for rosaceous crops, and a small number of *Osmia* species have been explored as commercial pollinators of apple, cherry, pear and plum (Batra 1978, Bosch and Kemp 1999, Ladurner et al. 2004, Monzón et al. 2004, Matsumoto et al. 2009, Sheffield 2014). In North America, *Osmia lignaria* Say (blue orchard bee) and *O. cornifrons* Radoszkowski (Japanese hornfaced bee) are the two species with the highest potential for use in

commercial orchards. *Osmia lignaria* is native to the USA, with two subspecies; *O. lignaria propinqua*, which is found in the west part of the country, and *O. lignaria lignaria*, which is present in the east (Rust 1974). *Osmia cornifrons* was introduced to the United States in the 1970s from Japan to be used as an apple pollinator (Batra 1978), and is now widely distributed in the eastern United States (Biddinger et al. 2009). Both are univoltine, solitary, cavity-nesting bees (Bosch and Kemp 2000, McKinney and Park 2012). In the wild, females nest in existing cavities made by wood-boring insects, but they can also nest in man-made structures such as wood blocks with drilled holes, cardboard straws, hollow reeds or bamboo (James and Pitts-Singer 2008, Sampson et al. 2009). Additionally, they have a natural tendency for building their nests in close proximity to other conspecific bees (Pitts-Singer 2007), facilitating their rearing.

Several strategies have been developed to rear and release mason bees in crops, but there are still issues related to how to improve the retention and reproductive success of these bees in commercial orchards (Sedivy and Dorn 2014). Especially considering that mass released, *Osmia* bees show high dispersal rates that can be as high as 50% (Torchio 1982b, Bosch 1994a). When bees are released as emerged adults the dispersal rates can reach 81% (Bosch 1994b). One way to reduce dispersal of young *Osmia* bees is to increase attractiveness of the nesting materials offered. This can be achieved by selecting the preferred nesting material and dimensions. Nesting preference studies for mason bees have shown that they preferred wood substrates over artificial substrates such as cardboard tubes, and preferred diameters between 6-9 mm for different species (Torchio 1982b, 1984, Rust 1998, Krunic and Stanisavljevic 2006, Biddinger et al. 2008, Sampson et al. 2009). Another way to increase attractiveness of substrates is by using chemical attractants that induce bees to nest in a specific location. Early studies recognized that several *Osmia* species prefer cavities from where they emerged over new cavities (Torchio

1984). Later studies showed that *O. lignaria* have an antennal response to fatty acids (decanoic acid, dodecanoic acid, and tetradecanoic acid) emitted from old cocoons (Pitts-Singer 2007) and are attracted to old nests by these volatiles (Pitts-Singer et al. 2016). Based on these results, a commercial *Osmia* attractant was developed from fatty acids isolated from *O. lignaria propinqua* nest-trapped in Utah. According to the patent, this attractant is expected to increase the number of nests of several *Osmia* species (e.g. *O. bicornis, O. bucephala, O. cornifrons, O. aglaia, O. ribifloris, O. bruneri*, and *O. cornuta*) (Pitts-Singer et al. 2016).

Despite existing knowledge of *Osmia* nesting preferences and management, there are still improvements needed for mason bees management in the field. These gaps include the effect of combinations of different shelters and nesting substrates, and the efficacy of the commercial attractant in the field for *O. lignaria* and other *Osmia* species. Variables such as the number and location of nesting sites, number of bee release sites, and effect of landscape are areas where more information is needed to improve the use of these bees as crop pollinators (Artz et al. 2013, 2014). Therefore, the aims of this study were to 1) elucidate the nesting substrate and shelter materials preferred by *O. lignaria* and *O. cornifrons* and 2) determine whether an attractant spray derived from *Osmia* cocoons is an effective way to increase the number of nests filled by *Osmia* female in a Michigan farm landscape setting.

METHODS

The experiments were conducted at two research centers of Michigan State University (MSU) during 2016. Both have experimental plots of tree fruit crops, including apple, cherry and plum; annual crops such as corn, and berries including blueberry and raspberry. Both centers also have small patches of woods and grassy areas, providing resources for *Osmia* bees.

For all experiments, boxes of 45 x 32 x 35 cm (length x width x height) with one open face were used as shelters. Inside each shelter, the nesting substrates were attached to a stake at the upper section so they were not in contact with any of the shelter walls. The open face of the box was covered with chicken wire (2.5 cm mesh size) to exclude birds and rodents (Fig. 2.1A-B). Shelters were attached to two metal posts and held approximately 1.5 m above the ground (Fig. 2.1A), with the open side facing east or southeast for exposure to sunlight early in the day (Torchio 1982a, 1982b, 1984). When more than one box was placed in a location they were at least 5 m away from each other. Each location was at least 60 m away from the others.

Osmia cocoons for release at the experiment locations were obtained from trap nests collected from the Horticultural Demonstration Garden at MSU (East Lansing, MI) in September 2015. Nests were opened and cocoons were extracted and sorted by sex based on size, since female cocoons are bigger (\approx 11 mm long) than male cocoons (\approx 7 m long) (Mader et al. 2010). Cocoons were stored in an environmental chamber at 4°C from November 2015, until their release. A sub-sample of 10 cocoons was taken from each batch once each week during February and March (2016), and emerged by holding them at laboratory conditions (\approx 25°C) to determine the proportion of each species and when they were ready to emerge quickly. For the experiments, bees were released as loose cocoons in cardboard emergence boxes (9.3 x 9.3 x 4.3 cm) with one 10 mm hole on one of the walls to allow bees to exit the box after emerging from the cocoons.



Figure 2.1. A) Wooden shelter with nesting tubes at Clarksville Research Center showing the basic layout of the shelters. B) Nesting substrates used in the shelter/substrate experiment, including the emergence box (upper left to right: 7 mm, 8 mm, 6 mm, 5 mm cardboard tubes, natural reeds; middle center: emergence box with exit hole; bottom center: wood block). C) Three bundles of 7 mm cardboard tubes used in the attractant spray experiment.

Shelters and nesting substrate study

This experiment was conducted at Trevor Nichols Research Center (TNRC), in Fennville, MI (USA) (42°35'38.26"N, 86° 9'18.03"W), between April-June 2016. For this experiment the variables evaluated were the shelter type and nesting substrates, both were tested together using a nested design. In this design, the main factor was the shelter type, with three levels corresponding to three shelter types: blue and white plastic totes (ULINE, Pleasant Prairie, WI)

and pine plywood boxes. The nesting substrates evaluated within each shelter type were a wood block with 48 holes (8 mm in diameter), natural reeds (*Phragmites* sp.) (7-10 mm inner diameter) and cardboard tubes with internal diameters of 5, 6, 7, or 8 mm. All the substrates were approximately 15 cm long. Wood blocks were closed at one end by a piece of cardboard, reeds were closed at one end by the natural node of the plant, cardboard tubes were closed at one end, and a paper straw open at both ends was inserted in each cardboard tubes each of 5, 6, 7 and 8 mm inner diameter were placed in each shelter. The relative placement of the substrates in the shelter was randomized, except for the wood block that was always placed in the lower section (Fig. 2.1B). The response variables were the proportion of the holes in each substrate used for nesting and the number of cells and female offspring per nest.

Ten locations were selected at TNRC for placement of *Osmia* nest shelters (Fig. 2.2). On April 19, 2016, one shelter of each of the three types was placed in a random order at each location. One emergence box was placed in the central shelter. In five locations the emergence boxes contained 60 female and 88 male cocoons that were at least 67% *O. cornifrons* (with the remaining being *O. lignaria*). In the remaining five locations the emergence boxes contained 55 female and 69 male cocoons that were at least 73% *O. lignaria* (with the remaining being *O. cornifrons*). These numbers were calculated to have at least 40 females of the desired species emerging at each location.



Figure 2.2. Shelter locations at Trevor Nichols Research Center at Fennville, MI. Magenta pins correspond to locations where *O. cornifrons* was released, yellow pins correspond to locations where *O. lignaria* was released.

Shelters were checked weekly to count the number of nest cavities that had been filled by nesting *Osmia* bees. Those with mud caps at the entrance of the cavity were considered completed. When the number of completed nests stopped increasing, in early June, shelters were brought back to the MSU Entomology Research Center (Lansing, MI) and kept at outside

ambient temperature until September 2016, when bees have finished their development into adults. Nests were then opened and the number of nests filled per treatment, number of cells per nest tube, and the number of females per nest tube were determined. One cocoon per nest tube was opened and the bee inside was placed in a jar with cyanide to kill the bee, and then pinned in order to identify the species that built each nest using the key by Arduser (2009).

Attractant to increase Osmia nesting

The attractant spray experiment was carried out at the Clarksville Research Center (CRC) in Clarksville, MI (USA) (42°52'17.92"N, 85°15'29.29"W), between April-June 2016. For this experiment, I evaluated whether application of an attractant spray to the nesting substrate affected the number of cavities filled by *Osmia* bees, and the number of cells and females produced per nest tube. The attractant spray consisted of decanoic acid (CH₃(CH₂)₈COOH) provided by Dr. Theresa Pitts-Singer of the USDA-ARS in Logan, Utah. To prepare the attractant, 5.8 g of decanoic acid was dissolved in 100 ml of ethyl acetate. The solution was transferred to a spray bottle that delivered 0.22 ml per spray, corresponding to 50 cocoons equivalents (CE) per application.

For this experiment, I used pine plywood shelters built by the author and 7 mm cardboard tubes as the nesting substrate. Each shelter contained three bunches of 50 tubes each (150 tubes per shelter) (Fig. 2.1C). Eight locations were selected at CRC (Fig. 2.3), and two shelters were placed at each location on April 26, 2016. Tubes in one shelter per pair were treated with the attractant, while tubes in the second shelter were left untreated. Treated tubes were sprayed with the attractant 24 hours before being placed in the field, and were kept apart from the untreated

tubes to avoid cross contamination. Each bundle of tubes was sprayed once, for a total of 1 CE per tube and 150 CE per shelter.



Figure 2.3. Shelter locations at Clarksville Research Center, Clarksville, MI. Magenta pins correspond to locations where *O. cornifrons* was released, yellow pins correspond to locations where *O. lignaria* was released.

Shelters were checked weekly to count the number of nest tubes filled during that week in each shelter. When the number of completed tubes stopped increasing around early June, boxes were brought back to the MSU Entomology Research Center (Lansing, MI) and stored until bees completed development and nesting activity quantified using the same methods as described above in the shelter/substrate experiment.

Data analysis

Shapiro-Wilk tests and diagnostic graphs (qq plots and residual plots) were used to assess data normality, and a Bartlett test was used to assess the homogeneity of variance among treatments (Shanks and Hutton 1976). Non-normal data were square root transformed prior to analysis. A generalized linear mixed model (GLMM) with beta distribution and logit link function in PROC GLIMMIX in SAS version 9.4 (SAS Institute 2016) was used to determine the effect of the shelter and substrate as well as the interaction between the two factors. If any of the factors was significant (p < 0.05), Tukey's test was used to determine the significance of differences among treatments. An analysis of variance (ANOVA) was used to determine whether shelter order at the location (left, center or right) affected nesting, using R version 3.1.1 (R Development Core Team, Vienna, Austria). For the analysis of the number of cells and females per nest, only the data from treatments where more than four nests were obtained were used. For O. cornifrons this included data from the following treatments (shelter/substrate): blue-5 mm, blue-6 mm, bluenatural reeds, wood-5 mm, wood-6 mm, wood-7 mm, wood-natural reeds, white-natural reeds. For O. lignaria this included data from the following treatments: blue-natural reeds, blue-wood block, white-natural reeds, wood-natural reeds.

To test the effect of the attractant, I only included data from locations where bees nested. This resulted in four replicates for *O. cornifrons* and three replicates for *O. lignaria*. To compare the number of nests obtained and the number of cells and females per nest in each treatment I used the non-parametric Mann-Whitney test, given that the data did not respond to transformations. For *O. lignaria*, given that there were only three replicates, I used the Kruskal– Wallis test to compare the number of nests obtained, and it was not possible to perform a
statistical test on the number of cells per nest because there was only one nest in all of the nospray shelters.

RESULTS

Shelter/substrate experiment

Seventy one percent of the nests obtained in this experiment belonged to *O. cornifrons*, and 29% to *O. lignaria*. The sex ratio for both species was 1.2 males per female. The order of the shelters at the location (left, center or right) did not affect the number of nests obtained, for *O. cornifrons* $(F_{(2, 12)} = 1.215, p = 0.331)$ or *O. lignaria* $(F_{(2, 12)} = 0.752, p = 0.492)$. More *O. cornifrons* nests (7.2 fold) were obtained in the location where this species was released $(F_{(1, 3)} = 10.76, p = 0.046)$, but the number of *O. lignaria* nests was not different between locations regardless whether it was released there or not $(F_{(1, 3)} = 3.901, p = 0.143)$ (Fig. 2.4).



Figure 2.4. Total number of nests obtained in locations where the target species was released or not for the shelter/substrate experiment at Fennville, MI. A) *O. cornifrons*. B) *O. lignaria*. Bars with the same letter are not significantly different, p > 0.05.

For *O. cornifrons*, there was a significant effect of the shelter type $(F_{(2, 15)} = 5.36, p = 0.0175)$, nest tube substrate $(F_{(5, 15)} = 9.12, p = 0.0004)$, and the interaction between the two factors $(F_{(2, 15)} = 4.87, p = 0.0235)$ on the percentage of cavities used. When splitting groups by shelter type, in the wooded and blue shelters, natural reeds were the most used substrate (wooden-reeds: 20 ± 7 %, blue-reeds: 10 ± 3 %) (mean \pm SE) (all p < 0.05), followed by 5 mm (wooden-5 mm: 4 ± 2 %, blue-5 mm: 3 ± 2 %) and 6 mm tubes (wooden-6 mm: 4 ± 2 %, blue-6 mm: 4 ± 2 %) (Fig. 2.5 A and B). In white shelters, all substrates showed a low use percentage close to 1% (all p > 0.05) (Fig. 2.5B).



Figure 2.5. Percentage of cavities of each substrate used by *O. cornifrons* in each shelter type, A) wooden, B) blue, and C) white shelters. Natural reeds and 5 mm cardboard tubes are the substrates with the higher proportion of use. Bars within a graph with the same letter are not significantly different, p > 0.05.

When splitting groups by substrate, *O. cornifrons* used 8.6% more reeds when they were in wooden shelters (reeds-wood: 13.4 ± 3.8 %) than when they are in blue shelters (reeds-blue: 4.8 ± 1.6 %) (all p < 0.05) (Fig. 2.6 F). The percentage of cavities used for the rest of the substrates did not change among the shelter types (all p > 0.05) (Fig. 2.6 A-E).



Figure 2.6. Percentage of cavities in each shelters type used by *O. cornifrons* for the different substrates. A) 5 mm tubes, B) 6 mm tubes, C) 7 mm tubes, D) 8 mm tubes, E) wood block, F) natural reeds. Bars within a graph with the same letter are not significantly different, p > 0.05.

For *O. lignaria*, there was no significant effect of the shelter type ($F_{(2, 8)} = 0.57$, p = 0.587), a significant effect of the substrate materials ($F_{(3, 8)} = 1.94$, p = 0.202) and no significant interaction between the two factors ($F_{(1, 8)} = 0.63$, p = 0.452). Natural reeds were the most used substrate (8.5 ± 1.1 %) (Tukey test, p > 0.05), with few individuals nesting in the other options available (Fig. 2.7).



Figure 2.7. Percentage of cavities of each substrate used by *O. lignaria*. Natural reeds is the substrate with the higher proportion of use. Bars with the same letter are not significantly different, p > 0.05.

For the number of cells per nest for *O. cornifrons*, there was no significant effect of the shelter type ($F_{(2, 183)} = 0.698$, p = 0.498), a significant effect of substrate ($F_{(3, 183)} = 5.964$, p = 0.0006) and no significant effect of the interaction between the two factors ($F_{(2, 183)} = 0.559$, p = 0.572). Among the substrates include in the analysis, 7 mm cardboard tubes and natural reeds

had higher number of cells per nest (6.0 \pm 1.7 individuals/nest and 6.0 \pm 0.2 individuals/nest, respectively) than the other sizes (all p < 0.05) (Fig. 2.8A). Shelter type did not significantly affect the number of females produced per nest for *O. cornifrons* (F_(2, 183) = 0.485, p = 0.616), there was a significant effect of substrate (F_(3, 183) = 8.096, p < 0.0001) but no significant interaction between the two factors (F_(2, 183) = 1.735, p = 0.179). On average, one female more per nest was produced in the 7 mm tubes (2.0 \pm 0.1 females/nest) and reeds (3.3 \pm 0.4 females/nest) than in the 5 mm (1.3 \pm 0.6 females/nest) and 6 mm tubes (1.8 \pm 0.5 females/nest) (Fig. 2.9A).



Figure 2.8. Number of cells build per nest in the most used substrates for A) *O. cornifrons*, and B) *O. lignaria*. Bars with the same letter are not significantly different, p > 0.05.

Osmia lignaria had a similar number of cells per nest in both natural reeds (7.4 ± 0.2 cells/nest) and wood blocks (8.6 ± 1.0 cells/nest) (Fig. 2.8B), with no significant effect of the shelter type ($F_{(2, 65)} = 0.533$, p = 0.589), or substrate material ($F_{(1, 65)} = 0.429$, p = 0.514). I was unable to include the interaction in the model since almost all nesting occurred in the natural

reeds. Regarding the number of females produced per nest for *O. lignaria*, there was no significant effect of the shelter ($F_{(2, 65)} = 0.187$, p = 0.829), no significant effect of substrate ($F_{(1, 65)} = 1.148$, p = 0.287) and was not possible to include the interaction in the model since almost all nests where obtained in the natural reeds, obtaining 3.1 ± 0.3 females/nest for natural reeds and 2.0 ± 0.8 females/nest for wooden blocks (Fig. 2.9B).



Figure 2.9. Number of females produced per nest in the most used substrates for A) O. *cornifrons*, and B) O. *lignaria*. Bars with the same letter are not significantly different, p > 0.05.

Attractant spray experiment

In this experiment, 86% of the nests obtained belonged to *O. cornifrons*, and 14% to *O. lignaria*, similar to the experiment reported above. I found on average 17 more *O. cornifrons* nests in locations where I released this species, but in this case the differences where not significant (X^2_1 = 1, p = 0.317) (Fig. 2.10A). For *O. lignaria* the total number of nests was similar in locations

where it was released or not ($X^2_1 = 1$, p = 0.317) (Fig. 2.10B). The sex ratio for *O. cornifrons* was 1.7 males per female, while for *O. lignaria* it was 1.0 male per female.



Figure 2.10. Total number of nest obtained in locations where the target species was released or not in the attractant spray experiments at Clarksville, MI. A) *O. cornifrons*. B) *O. lignaria*. No significant difference was found between treatments.

For *O. cornifrons*, I obtained six-fold more nests in the shelters treated with the attractant lure (15.0 ± 4.9 nests/shelter) than in the untreated shelters (2.0 ± 1.1 nests/shelter), (W = 2.0, p = 0.035) (Fig. 2.11A). There were no significant differences between treatments in the number of cells built per nest (spray: 7.1 ± 0.2 cells/nest; no spray: 8.4 ± 0.7 cells/nest) (W = 172, p = 0.193) or the number of females produced per nest (spray: 2.2 ± 0.2 females/nest; no spray: 2.1 ± 0.4 females/nest) (W = 259.5, p = 0.83) (Fig. 2.12).



Figure 2.11. Number of nest build per shelter when the nesting materials are sprayed or not with decanoic acid. A) *O. cornifrons* and B) *O. lignaria*. Bars with the same letter are not significantly different, p > 0.05.

For *O. lignaria* there were no significant differences in the number of nests obtained between treatments, although there was a small increase in the number of nests per shelter when the attractant was used (spray: 3.3 ± 2.8 nest/shelter; no spray: 0.1 ± 0.0 nest/shelter) ($X^2_1 = 1.5$, p = 0.221) (Fig. 2.11B). It was not possible to compare the number of cells and females per nest given that only one nest in the no spray treatment was obtained.



Figure 2.12. A) Number of cells per nest and B) number of females per nest build by *O*. *cornifrons* when the nesting materials are sprayed or not with decanoic acid. Bars with the same letter are not significantly different, p > 0.05.

DISCUSSION

Different nesting shelters and substrates can be more attractive than others for *Osmia* bees, and can influence the number of cells made and the number of females produced per nest. For *O. cornifrons,* when given a choice among different materials, the shelters and substrates with the highest use percentage were blue and wooden shelters with natural reeds and 5-6 mm cardboard tubes. In the case of *O. lignaria*, bees nested almost exclusively in natural reeds, regardless of the shelter type.

Earlier studies found that the percentage of cavities utilized is between 10-20 percent when bees are mass released in apple orchards (Torchio 1982a, 1982b, Yoon et al. 2015). This is similar to what I found in the wooden shelter/natural reeds treatment. Torchio (1982b) found that *O. lignaria* nest more in wooden materials than in milk cartons, which also coincides with observations in this experiment. Bosse et al. (2014) also report that *O. bicornis* (L.) prefer to nest

in reeds rather than in paper tubes. In this study I also found the typical male-biased sex ratio that is usually reported for *Osmia* species (Torchio and Tepedino 1980), as well as the typical range for the number of cells per nest (1-10 cells/nest) (Bosch and Kemp 2002, Mader et al. 2010).

Initial studies into Osmia nesting biology focused on how attractive different nesting materials are for bees (Torchio 1982a, 1982b, 1984, Bosch 1994b), followed by studies regarding the influence of different length and diameter of the cavities on bees preferences (Rust 1998). More recently, studies have addressed the effect of shelter color (Artz et al. 2014) as well as the number of shelters and cavity density for improved bee retention and reproductive success (Artz et al. 2013). However, few studies have addressed the interaction between shelter color/material and substrate type. In that sense, this study provides a new perspective for the strategies that can be used to manage Osmia bees in orchards. My research supports the conclusion that wooden substrates are the most attractive nesting materials for mason bees (Mader et al. 2010, MacIvor 2016). However, cardboard tubes are easier to process to obtain cocoons, it is possible to reuse them several times, and they are less expensive than wood materials or natural reeds, so they could be more suitable for mass productions of mason bees. Other studies have also shown a sufficient nesting success when using 7 mm diameter cardboard tubes (Artz et al. 2013, 2014). Another point to consider is that, because wooded blocks were in the base of the box, they had a higher chance of remaining wet after rain, possibly decreasing the attractiveness of this substrate, something that was also observed by Artz et al. (2013).

Some authors report that the color and patterns of shelters are important because it can be used by bees as a visual cue (Fauria and Campan 1998, Biddinger et al. 2008, Mader et al. 2010). Artz et al. (2014) found more *O. lignaria* nests and more females per nest in blue plastic boxes than in orange or yellow boxes. I also found differences in the rate of substrate use for the

different shelter types for *O. cornifrons*, particularly for natural reeds, for which wooden shelters yield a higher use proportion. It is possible that the combination of wood materials makes this arrangement more attractive to bees because it resembles their natural nesting environment. In the case of *O. lignaria*, I did not find a significant effect of the shelter type, but given the relatively small percentage of *O. lignaria* nests obtained, it is possible that the results observed could change if there was a larger number of females nesting in the trap nests. Other factors that can affect *Osmia* nesting success include the cavity density per shelter, as *O. lignaria* has been shown to prefer lower densities of tubes per box (Artz et al. 2013, 2014), the high dispersion rate when bees are mass released, the distance of the nests from the release site, and the response to weather conditions (Torchio 1982a). Additionally, interactions between *Osmia* species such as competition for nesting sites remains an under-studied aspect of their biology.

In this study, the number of cells or females per nest of *O. cornifrons* did not vary among shelter types, however, bees showed a strong preference for 7 mm cardboard tubes and natural reeds, with higher numbers of completed cells and a greater investment in females per nest in these nest resources. Artz et al. (2014) found that more *O. lignaria* females are produced per nest tube in blue plastic shelters than in orange or yellow shelters. However, in this case I did not find differences among shelter type, perhaps due to the low numbers of nests of *O. lignaria* obtained. Several authors report that nest cavities with greater diameters can promote more females per nest (Levin 1966), because for many *Osmia* species, females are bigger than males, so they are mainly produced in cavities of greater diameter. There is an upper limit to this, however, when the tube becomes too wide to be accepted ($\approx 12 \text{ mm}$) (Bosch and Kemp 2001). Given that in this experiment, *Osmia* bees mainly nested in natural reeds, few other substrates could be included in the analysis, but a similar pattern was observed for *O. cornifrons*, in which the substrates with

the wider diameter (reeds 8-10 mm) had more cells and more females per nest. The type of shelter can also affect the number of cells per nest for *O. lignaria* (Torchio 1982a, 1982b).

Several authors have found evidence that *Osmia* species rely on volatiles for nest location and recognition. Some studies showed that *O. lignaria* females use chemical cues produced by the Dufour's gland to mark and recognize their nests (Guedot et al. 2006, Pitts-Singer et al. 2012). *Osmia lignaria* is also more attracted to old previously used cavities than new ones (Torchio, 1984a), apparently driven by volatiles emitted by the old cocoons (Pitts-Singer 2007). The nesting-site selection behavior indicates that females tend to come back to the location where they emerge, and then scout several cavities, entering and exiting them, until a cavity is selected, with old cavities showing a higher probability of been selected (Levin 1966, Torchio 1984). Although not clear yet, this behavior suggests that females use the volatiles from the old cocoons as a local cue to come back to the location where they originally emerged, and then select a cavity from the options available. This suggests that the decanoic acid is not functioning as an attractant, but rather as an arrestant and identifier of nest tube locations, to increase the acceptance of holes for nesting by females. There is no direct evidence of this being a long-range attractant that would attract bees from downwind to orient towards a nesting location.

In this study I found that decanoic acid can increase the number of *O. cornifrons* females nesting in a location, as reported by the patent (Pitts-Singer et al. 2016), making this semiochemical a good option to increase retention of this species when mass released. The fact that decanoic acid did not affect the number of cells or the number of females per nest indicates that this molecule does not influence the investment that females make in their nests. In the case of *O. lignaria*, it is possible that bees did not nest much in any of the treatments because the nesting substrate offered was cardboard tubes, which based on the shelter/substrate experiment

described above, is not a preferred substrate. It is also possible that as this molecule was identified in cocoons of the western subspecies (*O. lignaria propinqua*), it could be less attractive for Michigan populations that belong to the eastern subspecies (*O. lignaria lignaria*). Although Pitts-Singer et al. (2016) report that the attractant is efficient for several *Osmia* species, further evaluations are needed to determine the range of species with which this attractant will be effective. It is also important to test whether this or future lures can also attract bee natural enemies, as Glasser and Farzan (2016) showed that *Melittobia acasta* (Hymenoptera: Eulophidae), an *Osmia* parasitoid, can be attracted to *O. lignaria propinqua* cocoons.

My results highlight the potential of the two *Osmia* species to be used as alternative pollinators in Michigan fruit crops. The higher success rate of *O. cornifrons* and its better response to the attractant spray suggests that this bee is easier to manage than *O. lignaria*. However, the use of non-native species for pollination should be carefully considered, given that in many cases they transmit diseases and parasites (Goulson and Hughes 2015), compete with native species for resources (Thomson 2004, 2006), and can pollinate invasive species (Hanley and Goulson 2003). Further, many studies have shown that *O. lignaria*, a native species, is a promising species and it is possible to manage them in commercial settings. This reinforces the importance of continuing to explore strategies to improve the retention and reproductive success of mason bees in agricultural landscapes. The knowledge about preferred trap nest for bees can be also used to increase populations of *Osmia* spp. in natural areas with conservation goals, or to increase natural populations that support pollination service (Stubbs et al. 1997).

CHAPTER 3. SOIL USE BY *O. lignaria* and *O. cornifrons* (HYMENOPTERA: MEGACHILIDAE) IN AN AGRICULTURAL ENVIRONMENT

INTRODUCTION

Most bee species (Hymenoptera: Apoidea) build their nests underground, excavating tunnels and building cells where they deposit pollen and nectar to feed their larvae (Roubik 1989, Michener 2007). However, many bee species in the families Apidae and Megachilidae nest above-ground (Linsley 1958, Krombein 1967). Nests can be built in cavities between rocks (e.g. Euglossini), preexisting cavities in wood (e.g. Megachilini, Osmiini), excavating tunnels in decaying wood (e.g. Xylocopini), in abandoned mammal burrows (e.g. Bombini), or on trees (e.g. Apini, Meliponini) (Linsley 1958, Roubik 1989, Torchio 1989, Nates-Parra and Gonzalez 2000, Nates-Parra 2001, Gonzalez et al. 2004, 2009, Marchi and Melo 2010). Studies on the nesting preferences of solitary bees have been relatively scarce (Levin 1966, Torchio 1982a, 1982b, Cane 1991, Wuellner 1999, Cane et al. 2007). In recent years more attention has been given to this topic, due to increasing interest in having alternative pollinators for crops, including studies of ground nesting and stem nesting bees (Klein et al. 2007, Christmann and Aw-Hassan 2012, Sedivy and Dorn 2014).

Bees in the genus *Osmia* (Megachilidae) are univoltine (one generation per year), solitary, and cavity-nesting (Levin 1966). In the wild, mason bees nest mainly in soil or existing cavities made by wood-boring insects, but they can also nest in man-made structures like wood blocks with drilled holes, cardboard straws, hollow reeds or bamboo (Levin 1966, Bosch and Kemp 2002, Cane et al. 2007). Most species use a mix of plant materials and mud to build the partitions between cells (e.g. *Osmia kenoyeri* Cockerell, *Osmia unca* Michener), but some

species exclusively use soil to build the partitions (e.g. *Osmia lignaria* Say and *Osmia cornuta* Latreille) (Linsley 1958, Cane et al. 2007).

For the species that use only soil for partitions, it is common to find females congregating at mud collecting sites where the soil is wet, with several females taking soil from the same excavated hole (Torchio 1989). Females gather mud by scraping with the mandibles and forelegs, then shaping it to create a round pellet 2-3 mm in diameter using the forelegs, mandibles, and the ventral surface of the posterior tip of the abdomen. She then holds the pellet between her mandibles and flies back to the nest (Levin 1966, Raw 1972). The soil is deposited as a foundation that circumscribes the inner wall of the nest. Additional soil is added in concentric circles around the foundation until a solid wall is formed across the nest (Torchio 1989). Females can spend 2 - 4 minutes depositing each mud ball into the nest (Levin 1966). A total of 8 to 12 mud collecting trips are necessary in order to have enough soil for one cell partition, requiring about 20 min to build each partition (Torchio 1989, McKinney and Park 2012).

Some of the species that collect exclusively soil as a material for cell partitions are particularly important for agriculture. *Osmia cornifrons* (Radoszkowski) has been used as an managed orchard pollinator in Japan since the 1940s, and was introduced into United States in 1977 for crop pollination (Batra 1978). *Osmia lignaria* (native to the USA), *Osmia bicornis* (L.) and *O. cornuta* (Latreille) (native to Europe) have been proposed as alternative commercial pollinators for rosaceous tree crops, given their strong preferences for this group of plants and their high efficiency at pollinating them (Bosch and Kemp 2000, 2002, Vicens and Bosch 2000b, Ladurner et al. 2004, Gruber et al. 2011, Sheffield 2014).

Most studies on the nesting preferences of stem nesting bees, including *Osmia*, have focused on the kind of preferred cavities and shelters, effect of cavy density and release density (Sampson et al. 2009, Artz et al. 2013, 2014, Yoon et al. 2015). Even though soil is an essential and limiting resource for many bee species (both ground nesting bees and the ones that use it for cell partitions) (Cane 1991, Krunic and Stanisavljevic 2006), few studies have investigated what parameters of the soil (texture, structure, moisture level, organic matter content, etc.) are important for bees when selecting a nesting site or soil collecting sites. Soil texture, defined as the percentage of clay (< 2 μ m particles), silt (3-50 μ m particles) and sand (51-2000 μ m particles) in a soil sample (Foth 1990), is one of several factors that can influence nest site selection of ground nesting bees and wasps (Krombein 1967, Cane 1991, Scott 2016), so it is possible that it will also play a role in nest construction by *Osmia* species.

Both *O. lignaria* and *O. cornifrons* are present in Michigan, where they are effective pollinators of important fruit crops such as apples, cherries and pears (Bosch and Kemp 1999, Ladurner et al. 2004, Monzón et al. 2004, Matsumoto et al. 2009, Sheffield 2014). Determining the type of soil these bees use in agricultural landscapes should provide avenues for increasing their role as alternative pollinators. Understanding the soil that these bees prefer may also improve our ability to maximize their propagation, by ensuring that soil is not a limiting factor. *Osmia* bees are able to make choices over the plants they visit for pollen (Monzón et al. 2004, Bosch et al. 2006, Sheffield et al. 2008), and the substrates they use for nesting (Torchio 1982a, 1984, Artz et al. 2013, Chapter 2), so I expect that they can also select soil from the options available to them. However, it is not known how selective they may be in soil selection and whether they only select from the immediate soil type or will fly further for their preferred type. Given this, the objectives of this study were to 1) describe the texture of the soil used by *O*.

lignaria and *O. cornifrons* in an orchard setting and 2) explore whether the soil used by these *Osmia* bees is similar to or distinct from the characteristics of the soil surrounding the nest.

METHODS

The experiment was conducted at Trevor Nichols Research Center (TNRC), in Fennville, MI (USA) (42°35'38.26"N, 86° 9'18.03"W), between May-June 2016. The station has experimental plots of tree fruit crops, including apple, cherry and plum; annual crops such as corn, and berries including blueberry and raspberry, as well as patches of woods and grassy areas. There are small streams and side ditches that can provide wet soil for bees. A surface (0-25 cm) soil texture map of the station and surrounding areas was generated using the USDA Soil Survey website (https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm). According to this map (Fig. 3.1A), and the pesticide application plan of the station, 12 locations were selected that provided a variety of soil texture conditions and low risk of pesticide exposure. At each location, one bee shelter was installed. Nesting shelters consisted of pine plywood boxes (26 x 28 x 28 cm) with an open face. Inside each shelter, I placed 50 bamboo stems, approximately 15 cm long with an inside diameter of 7-10 mm. Shelters were held 1.5 m above ground by a metal post, and the open face of the shelter was covered with chicken wire (2.5 cm mesh size) to exclude birds and rodents (Fig. 3.2).



Figure 3.1. Maps of the Trevor Nichols Research Center near Fennville, MI, showing: A) surface soil (0-25 cm) map generated with the USDA Soil Survey online database in relation to the locations of shelters for *Osmia* bees. B) measured soil texture of surface samples (0-10 cm) taken from the research center. The estimated area available for bee foraging depicted in the figure was based on a 250 m foraging range from each shelter location. Alternative locations correspond to *Osmia* shelters in the stations installed for other experiments, from where nests were taken to increase the number of soil samples from bees' nests.

At each shelter, I placed an emergence box containing 50 female and 60 male loose *Osmia* cocoons. Female cocoons were 73% *O. lignaria* and 27% *O. cornifrons*, while male cocoons were 88% *O. lignaria* and 12% *O. cornifrons*. The emergence box consisted of a

cardboard box of 9.3 x 9.3 x 4.3 cm, with a hole on one face to allow bees to leave the box after emergence. Cocoons were gathered from trap nests located at Michigan State University (MSU) campus in East Lansing, MI, in September 2015. Nests were opened and cocoons were extracted and sorted by sex based on size, since female cocoons are bigger (\approx 11 mm) than male cocoons (\approx 7 m) (Torchio and Tepedino 1980). Cocoons were stored in an environmental chamber at 4°C from November 2015 until their release. A sub-sample of 50 cocoons was taken from each batch and emerged in the lab by holding them at laboratory conditions (\approx 25°C) to determine the proportion of each species.



Figure 3.2. Wooden shelters used at the research station, including the emergence box (bottom) and bamboo stems (top) offered to *Osmia* bees for nesting.

Soil sampling

To confirm the soil texture reported by the Soil Survey, soil samples were collected at the research stations and surrounding areas. The foraging range of *O. lignaria* and *O. cornifrons* is

between 30 to 150 m from the nest (Bosch and Kemp 1999, Monzón et al. 2004, Matsumoto et al. 2009, Biddinger, Joshi, et al. 2013), and they have been reported to collect soil from locations 54 m away from their nest (Torchio 1989), so an area of 250 m radius was established around each nesting shelter, to represent the area available for bees to forage (Fig. 3.1). The research station site was sampled using a total of 62 soil samples taken in and around the station, including locations in each of the areas with different texture reported by the Soil Survey. At each of the locations selected for soil samples, five subsamples from the upper 10 cm layer were collected in a 2 m radius from the selected point. The subsamples were then mixed to complete approximately 500 g of soil per sampling point.

To obtain soil samples from bee nests, bamboo tubes with a mud plug from each shelter where bees nested were brought back from the field after the nesting period was complete (approximately June 10, 2016). Nests were kept at the MSU Entomology Research Center (Lansing, MI) at outside ambient temperature until September 2016, when bees have finished their development into adults. The bamboo tubes were then opened and all soil partitions present in the nest were collected from 24 *O. cornifrons* and 22 *O. lignaria* nests. Partitions of the same nest were mixed and homogenized. Thirteen of the *O. cornifrons* nests and four of the *O. lignaria* nests came from alternative nest shelters placed in the station for other experiments, but that were close to the shelters used in this experiment (zone 2 and 3) (Fig. 3.1B). Additional tubes were opened to collect individual partitions from eight *O. cornifrons* nests (total 67 partitions) and six *O. lignaria* nests (total 44 partitions). In this case, each partition was kept apart from each other, and the order in which the partitions were in the nest was noted. To confirm the species that built each nest, one cocoon per nest was opened and the bee inside was

placed in a jar with cyanide to kill the bee, which was then pinned and identified using the key by Arduser (2009).

Particle size analysis

A particle size (texture) test was use to analyze soil samples. Soil samples from TNRC were analyzed using the hydrometer method at the Soil and Plant Nutrient Laboratory at the Department of Plant, Soil and Microbial Sciences of MSU. Samples were processed following the standard laboratory protocols. Briefly, 100 g of soil per sample was oven dried for 12 h, then 100 ml of 5 % sodium metaphosphate (NaO₃P) solution were added to disperse particles, that were then placed in a shaker overnight. The percentage of sand, silt and clay was then determined using the standard hydrometer methodology (Foth 1990).

Texture analysis of soil samples from *Osmia* nest and individual partitions was performed using the laser diffractometry technique at the Department of Geography, Environmental and Spatial Sciences of MSU. This technique allows texture analyzes of samples as small as 0.5 g, and the results are directly comparable to the results obtained by the hydrometer method (Miller and Schaetzl 2012). Samples were gently ground and dispersed by adding 2 ml of 5 % sodium hexametaphosphate ((NaPO₃)₁₃Na₂O) solution and shaken for 10 min. Samples were then run using a Malvern Mastersizer 2000E laser particle size analyzer with a Hydro 2000MU pump accessory (Malvern Instruments Ltd., Worcestershire, UK), following the laboratory protocols. This provided the particle size analysis of the soil samples in terms of percentage of clay, silt and sand.

Partition color analysis

Soil partitions from 40 *O. cornifrons* nests and 20 *O. lignaria* nest were sorted by color. Pictures of partitions with distinctive color patterns were taken using a Leica S8AP0 microscope with a MC120 HD camera attached. Partitions of the same color were ground and mixed together. Moistened samples were compared to a Munsell soil color chart to determine the color (hue, value and chroma). A subsample from each color was run for texture analysis using the diffractometry technique described previously.

Data analysis

A principal component analysis (PCA) with R version 3.1.1 (R Development Core Team, Vienna, Austria) was used to explore patterns in the composition of the nest partitions for the two species of *Osmia*, in relation to sand, silt and clay content. A generalized linear mixed model (GLMM) with beta distribution and logit link function was used. Nest tube was included as a random effect to take account of partitions from the same tube being more similar given than they were collected by the same females. For the GLMM, I used PROC GLIMMIX in SAS version 9.4 (SAS Institute 2016) to compare between species, and among cells and soil zones. For soil samples from the whole nest, each texture component (sand, silt and clay) was individually analyzed for differences between the two *Osmia* species, among the four soil zones, and the interaction effect. Soil from individual partitions was analyzed separately for each species, to determine whether the partition's position (as a ordinal variable) in the nest or the soil zone affected the soil composition, and whether there was an interaction between these factors. If these factors were significant, a Tukey test was used to separate the means (p < 0.05).

RESULTS

The soil samples taken in and around the research station showed different soil textures than the map generated with the online Soil Survey database (Fig. 3.1B). Only three of the six textures reported by the soil survey were actually present (loam, loamy sand and sandy loam), but two additional textures were found (sandy clay loam and clay loam). Based on the measured soil texture of the site, locations of the *Osmia* shelters were classified into four zones, depending on the soil texture near the nest location (Fig. 3.1B). In zone 2, which had mainly sandy clay loam texture, there were not enough *O. lignaria* nests for individual partition analysis, so only the whole nest analysis was conducted. Consequently, the individual partition analysis includes samples only from three zones, where zone 1 was mainly sandy loam, zone 3 was mainly sandy loam and loamy sand, and zone 4 that was mainly loamy sand.



Figure 3.3. Texture of soil samples from A) the whole nest and B) from individual partitions for *O. cornifrons* (red) and *O. lignaria* (blue). The clay content in *O. cornifrons* samples was significantly higher than in *O. lignaria* samples (p < 0.05).

When analyzing soil samples from complete nests, most of the samples of both species were classified as loam or sandy loam (Fig. 3.3A). The PCA shows a clear separation between the two species (Fig 3.4), with the analysis highlighting variation between the species based on only sand in the X axis, and clay in the Y axis. Silt composition was the least variable component of the soil and was not included in the PCA plot. These differences were mainly represented by an average 5.8% higher clay content in *O. cornifrons* samples ($F_{(1, 38)} = 12.77$, p = 0.001) (Table 3.1), without an effect of the location of the nests ($F_{(3, 38)} = 1.94$, p = 0.139) or an interaction between factors ($F_{(3, 38)} = 1.04$, p = 0.385). Silt and sand content in samples of both species were similar (all p > 0.05). Clay content in *O. cornifrons* samples had a slightly wider range (6.1 -20.9 %) than *O. lignaria* (2.1 – 15.2 %), with the opposite for the silt and sand content, for which *O. lignaria* showed a wider range than *O. cornifrons* (Table 3.1).



Figure 3.4. Biplot of the principal component analysis for the soil texture of whole nest samples. PC1 (sand) and PC2 (clay) are shown. Each sample is represented by one dot and the color label correspond to the species, *O. cornifrons* (red) and *O. lignaria* (blue).

Table 3.1. Soil texture (range and average \pm SE) of complete nests soil samples collected by *O*. *cornifrons* and *O*. *lignaria*. Means with the same letters within the same column are not significantly different (p > 0.05).

		Range			Average		
	n	Clay (%)	Silt (%)	Sand (%)	Clay (%)	Silt (%)	Sand (%)
O. cornifrons	24	6.1-20.9	23.5-45.1	36.7-69.9	14.1±0.9 a	34.1±1.2 a	51.9±1.7 a
O. lignaria	22	2.1-15.2	15.5-49.3	40.5-82.5	8.3±0.8 b	37.1±2.1 a	54.5±2.1 a

		Range			Average		
	n	Clay (%)	Silt (%)	Sand (%)	Clay (%)	Silt (%)	Sand (%)
O. cornifrons	67	2.1-34.2	20.3-76.3	3.6-77.7	12.4 ± 0.9	39.4 ± 1.4	48.2 ± 2.1
O. lignaria	44	1.5-27.8	19.6-86.5	0.0-78.8	8.9 ± 0.8	46.4 ± 2.9	44.7 ± 2.0

Table 3.2. Soil texture (range and average \pm SE) of samples from individual cell partitions collected by *O. cornifrons* and *O. lignaria*

When analyzing individual partitions of both species, the range of all particle components was wider than the whole nest samples (Table 3.2). Similar to the whole nest samples, the clay content range was wider for *O. cornifrons*, but silt and sand content ranges were wider for *O. lignaria* (Table 3.2). Due to this high variation, partitions were categorized as loamy sand, sandy loam, loam, silt loam or silty clay loam depending on the specific sample (Fig. 3.3B), although most of the samples were in the range of sandy loam and loam. It was also observed for both species that when considering partitions from the same nest, sometimes all the partitions were grouped and had a common texture, while in other nests each partition had different textures, varying mainly in sand content (Fig. 3.5).



Figure 3.5. Texture of soil samples from individual partitions of the nest of A) *O. cornifrons* and B) *O. lignaria*. Dots of the same color correspond to partitions taken from the same nest.

The PCA suggests similar soil composition for partitions in different positions in the bamboo nests (Fig. 3.6), and for *O. cornifrons*, neither the zone of the nest or the position of the partition in the nest (Fig. 3.7) had a significant effect on any of the texture components (clay:

zone $F_{(3, 54)} = 0.86$, p = 0.46, cell position $F_{(1, 54)} = 0.09$, p = 0.76; silt: zone $F_{(3, 54)} = 2.49$, p = 0.070, cell position $F_{(3, 54)} = 0.23$, p = 0.63; sand: zone $F_{(3, 54)} = 2.01$, p = 0.12, cell position $F_{(1, 54)} = 0.05$, p = 0.82). However, there was a marginally significant effect of the zone of the nest on the silt content of the partitions ($F_{(3, 54)} = 2.49$, p = 0.0704), with partitions of nests from zone 4 (mainly loamy sand) showing a 10 % lower silt content than those in zone 1 (mainly loam) (Fig. 3.8). Also, there was a significant interaction between factors ($F_{(3, 54)} = 3.23$, p = 0.029) for the clay content of the partitions. This significant interaction without a significant effect of the main factors was due to finding differences in the clay content between partitions only in zone 2 ($T_{(54)} = -3.21$, p = 0.0023) (Fig. 3.9). For *O. lignaria*, there were no effects of the positions of the partition in the nest, the zone of the nest or an interaction between factors on any of the texture components (all p > 0.05).



Figure 3.6. Biplot of the principal component analysis for the soil texture from individual partitions for A) *O. cornifrons* and B) *O. lignaria*. PC1 (sand) and PC2 (clay) are shown. Each sample is represented by one dot and the color label (cell) corresponds to the order of the partitions in the nest, with 0 being the most inner partition.



Figure 3.7. Mean ± SE of sand, silt and clay content of soil partitions for nests of A) *O*. *cornifrons* and B) *O*. *lignaria*.



Figure 3.8. Mean \pm SE percentage of clay, silt and sand in individual partitions from *O*. *cornifrons* nests from different zones in the research station. Silt content in partitions from zone 4 showed a trend towards higher particle size (p = 0.07).



Figure 3.9. Percentage of clay (mean \pm SE) in *O. cornifrons* partitions at the different zones in TNRC. Partitions showed significant differences in clay content (p < 0.05) only in zone 2.

Partitions from nests were sorted in six different colors, plus a "mix" category, when a partition showed more than one color (Fig. 3.10). Some of the colors, even when classified as different colors at first, had the same hue, value and chroma values (Color 1 and 4), but they had different texture. In other cases, they showed different hue, value and chroma values (Color 3 and 5), but they showed the same texture category (Table 3.3). Additionally, mixed color partitions, which account for approximately 8 % of the partitions for both species (Fig 3.11), presented a highly variable pattern of colors, from spirals and circular patterns that follow the construction configuration of the partition, to spotted color patterns (Fig. 3.10).



Figure 3.10. Different color patterns observed on the cell partitions of *O. cornifrons* and *O. lignaria*. Patterns can vary from spotted (A-C) to spiral or concentric (E-H). Soil of different colors suggest that they were collected at different location or at different depths in the same location.

	Color			Texture			
	hue	Value	Chroma	Clay (%)	Silt (%)	Sand (%)	Category
Color 1	2.5y	4	4	13.5	33.6	52.8	Sandy Loam
Color 2	2.5y	3	3	8.6	37.4	54.1	Sandy Loam
Color 3	10yr	4	6	12.4	38.2	49.4	Loam
Color 4	2.5y	3	3	9.4	38.6	52.0	Sandy Loam
Color 5	2.5y	4	4	13.9	36.6	49.5	Loam
Color 6	2.5y	3	2	10.7	40.4	48.9	Loam
Mix	2.5y	4	4	11.4	40.2	48.4	Loam

Table 3.3. Color and texture of the different partition's color identified in O. cornifrons andO. lignaria nests.



Figure 3.11. Percentage of partition of each color identified in A) *O. cornifrons* and B) *O. lignaria* nests

DISCUSSION

This study revealed variability with some species-specific differences in composition of soil use by a native and a non-native *Osmia* bee species. To the best of my knowledge, this is the first report of the texture of the soil collected by *O. cornifrons* and *O. lignaria*, and it sheds light on the soil preferences and ecological adaptations of these species. It also has to be stated that there could be Japanese literature regarding *O. cornifrons* that is not accessible to me. The few reports of soil texture preferred by mason bees indicate that they prefer soil that is soft and damp with a high clay content (Rau and Mo 1937, Medler 1967, Mader et al. 2010). However, these reports are usually based on anecdotal observations and there are no descriptions of the particle size composition. Although other soil factors not measured in this experiment, such as organic matter content or moisture content may influence soil selection by mason bees, soil texture is probably one of the most important variables driving *Osmia* soil selection, given that partitions and entrance plugs must be resistant enough to prevent parasites and predators to enter the nest, and soil texture has been found to be an important factor for bees nest site selection by several ground-nesting bee species (Cane 1991, Potts and Willmer 1997).

Using a unique application of laser diffractometry employed in the geological sciences, I found wide variation in the soil texture that bees bring back to the nest, indicating that they are flexible in the type of soil used to make nest partitions. The sand content in partitions ranged from 0.0 to 78%, but the clay content was never higher than 34%, with an average between 8-10%, so most of the samples can be classified as loam or sandy loam. Although these results contradict what was previously thought about mason bee soil preferences, it has to be considered that the soil in the area where bees were nesting was in the sandy spectrum, with a few locations presenting clayey texture. Given this, my results only indicate that *O. cornifrons* and *O. lignaria*

are able to use sandy soil to build the nest partitions. Experiments where bees have access to soil of several textures are needed to explore their soil preferences. Cage choice experiments where soil of different textures is offered to females to evaluate their preferences and constancy of soil use would give a better idea of their soil preferences. Cage experiments where only one type of soil is offered could help to elucidate the level of plasticity exhibited by these bees at the time of using soil of a specific texture. This type of experiment also would allow a more detailed manipulation of the soil offered.

In this study, I found that O. lignaria collect soil with a clay content 5.8% lower than O. cornifrons. Given than O. cornifrons is an introduced species from Japan, it may have evolved in places with sandier soil types than the ancestral home of O. lignaria. But the fact that it has been successful at establishing in North America (Biddinger et al. 2009), suggests that this species has sufficient plasticity to use different soils than the ones found in their native range. On the other hand, O. lignaria is a species that is widely distributed in North America (Rust 1974), also indicating that it has to be able to use a wide range of soil textures, given that the available soil resources change between geographical areas. One possible explanation for the average low clay content in the soil collected by bees is that soils with high clay content shrink when dry (Schoeneberger et al. 2012), so if bees build partitions with wet clayey soil, it could crack when dry, opening nests to parasitoids and predators. This soil may also be more challenging to collect and/or mold for mason bees, a question that could be answered through manipulative experiments. The fact that soil of relatively large particle sizes (sandy loam and loam) is used by these bees, suggests that females use a sticky substance to help keep soil particles together. If present, this substance is probably secreted by the salivary glands and present in bee' saliva, given that many other bees secrete substances to waterproof the nest or keep together building
materials, and even nectar could also be used as a sticky substance (Michener 2007). This mix of large particles in the mud and a substance that sticks them together could make partitions harder than if they were made with mud of a smaller particle size. Harder partitions could more efficiently prevent the entrance of parasites or natural enemies to the nest.

For both species of *Osmia* studied in this research, the range of soil textures used increased when considering individual partitions rather than the whole nest samples. This indicates that even when the general soil collected by both species is loam or sandy loam, they can collect soil from areas with a wide range in sand content. Interestingly, for both species, I found that partitions from the same nest can all have a common texture or a large variation in texture. This seems to indicate that some females collect soil from locations with different textures to build each partition, while other females collect soil of similar textures (from the same or different locations) to make all the partitions in the nest. This observation seems to indicate that within a population there are differences in the preferences and consistency of soil selection between individuals. However, a larger sample size is needed to determine whether this variability is common and to evaluate the role of available soil on the level of variation individual bees show. A hypothesis that could be tested is that if more preferred soil is offered, the level of variation among individuals will decrease.

Despite the variation among individual partitions, the percentage of the different texture components was relatively constant among zones and along the nesting period. The similar soils collected in different zones could be due to several factors. First, the soil in and around the research station turned out to have much less texture variability than expected based on the Soil Survey, with most of the soil in the sandy spectrum. So is not surprising that most of the samples collected by bees were sandy loam or loam. Second, it is possible that bees are flying more than

250 m to collect their preferred soil for the nest. The foraging range (for pollen and nectar) of these bees is between 50-150 m (Bosch and Kemp 1999, Dogterom 1999), but individual flights can extend beyond 600 m (Zurbuchen et al. 2010), making it possible that bees collected soil outside of our study area. Future studies should also include a larger sample size regarding the number of nests from where samples are taken, and a larger study area. Having a detailed and accurate soil map of the area would also help to determine the degree of correlation between the type of soil available and the soil that females bring back to their nests.

The effect of the soil in the area also needs further investigation, given that I observed a weak effect of the soil zone on soil texture that bees collected. Changes in clay content over time observed in zone 2 could be due to changes in the availability of wet soil in that region, given that soils of different textures vary in their water retention capacity. If a water source is not constant across this site (i.e. rain, rivers, lakes, crop irrigation), soils that retain water for longer periods (silty loam or clayey soils) would be available to be used by bees for longer periods than soil with lower water-holding capacity (dry, sandy soil). This highlights the importance of taking the hydrology and climate of a zone into account when considering mason bees as pollinators, since a lack of water sources could make it difficult for bees to use soil, even if adequate textures are in the area.

To build one partition, *Osmia* bees need to make 8-12 mud collecting trips (Torchio 1989), meaning that they can potentially collect soil from multiple locations for constructing each partition. The differences in soil color that I observed in single partitions may be due to differences in organic matter content or the presence of some elements that alter the soil color (Schoeneberger et al. 2012). The presence of areas of different colors in an individual partition suggests that bees collected soil from more than one location to build one single partition, or that

they dig into different layers of soil in the same place as they collect sequential soil loads. The relationship between color and texture of the samples is not clear, as two samples of the same color can have different textures, so it is also possible that partitions of a uniform color are made of soil collected at different locations. This makes it difficult to establish correlations between the soil in an area and the soil found in individual partitions or nests, given that partitions are created by a mix of soil from different locations, and in the same way, soil samples from the whole nest are a mix of several partitions. On the other hand, if females coming back to the nest with mud balls were captured, these samples would be too small to run a texture analysis. This makes it necessary to develop a different approach that could give information on where bees are collecting soil, such as tracking bees to the mud collecting sites or analyzing the soil from locations where females have been observed collecting soil.

Despite these remaining gaps in our understanding of soil use by *Osmia* bees, data from the present study provide an important first step for understanding the soil use and preferences of these insects, showing that they use soil from a broader range of textures than previously thought. I also found that *Osmia* species and individuals vary in the type of soil collected, with females even bringing mud from different locations to build one single partition. Future studies of soil collected by *Osmia* should also take into account variables such as organic matter content, moisture content and bulk density. Future studies could also address differences in soil preferences between different populations and subspecies of *O. lignaria*, given that phenological differences have been already shown between the subspecies (Pitts-Singer et al. 2014), and this information will be relevant when planning to use these bees as orchard pollinators in regions with different soils.

CHAPTER 4. POLLEN USE BY *Osmia lignaria* (HYMENOPTERA: MEGACHILIDAE) IN HIGHBUSH BLUEBERRY

INTRODUCTION

Bees in the genus *Osmia* (Megachilidae) are an important group of pollinators with potential for pollinating fruit crops (Bosch and Kemp 1999, Monzón et al. 2004, Matsumoto et al. 2009). These univoltine, solitary, cavity-nesting bees are commonly known as mason bees because some species use mud to make the partition between cells (Bosch and Kemp 2000). *Osmia lignaria* Say is a species native to North America that emerges early in the spring and has a strong preference for plants in the family Rosaceae, including crops such as apple, cherry, pear and plum (Bosch and Kemp 1999, Ladurner et al. 2004, Monzón et al. 2004, Matsumoto et al. 2009, Mader et al. 2010, Sheffield 2014). They are considered more efficient pollinators than honey bees for this group of plants because they mainly forage for pollen, constantly make contact with the reproductive parts of the flowers, and tend to gather pollen from at least two cultivars during the foraging trips, promoting cross pollination (Bosch and Kemp 2001, Matsumoto et al. 2009, Matsumoto et al. 2009, Matsumoto and Maejima 2010).

In the United States, *O. lignaria* is commercially available in some areas (Bosch and Kemp 2000, 2002, Mader et al. 2010), and its emergence can be manipulated by controlling temperature and keeping dormant cocoons in cold storage until the target crop bloom or by breaking the dormancy period by increasing temperature (Mader et al. 2010). Due to this, *O. lignaria* has been successfully used as a pollinator of other crops whose bloom period do not match with the natural flying period of this bee, such as almonds (Artz et al. 2013, 2014).

Highbush blueberry (*Vaccinium corymbosum*) is an economically important crop that requires insect pollination due to its poricidal anthers, dense pollen, and floral morphology that promotes cross-pollination. Additionally, some varieties are not self-compatible or show a significant increase in fruit quality when cross-pollinated (McGregor 1976, Ritzinger and Lyrene 1999). Bumblebees (*Bombus* spp.) are recognized as some of the best pollinators of this plant given their ability to "buzz-pollinate" the flowers (Stubbs and Drummond 1997, Brevis 2001, Mackenzie 2009, Scott 2016). *Osmia* bees can also visit and pollinate blueberries (Dogterom 1999, Sampson and Cane 2000, Cutler et al. 2015). *Osmia lignaria* has been found in 55% of 80 blueberry sites sampled in British Columbia, Canada, (Dogterom, 1999), and nine *Osmia* species have been reported to visit blueberries in Maine, but with low relative abundance (Bushmann and Drummond, 2015). In southwest Michigan, *Osmia* species can be observed in blueberry fields during the pre-bloom and bloom periods (Tuell et al. 2009), suggesting that these bees have potential for pollination of blueberries.

Several species of *Osmia* have been tested in controlled experiments (greenhouse or cage) for their ability to pollinate blueberry, and positive results have been obtained for *O. lignaria, O. atriventris* Cresson, *O. cornifrons* Radoszkowski and *O. ribifloris* Cockerell regarding transferring pollen for different *Vaccinium* species, in some cases achieving the same level of pollination as bumblebees (Stubbs and Drummond 1997, Dogterom 1999, Sampson and Cane 2000, Wilkaniee and Giejdasz 2003, Sampson et al. 2004, West and McCutcheon 2009). Dogterom (1999) reports that in blueberry fields in British Columbia, 86% of *O. lignaria* females captured at the nest entrances had blueberry pollen on their body. However, other reports show that fruit of rabbiteye blueberry (*V. ashei*) pollinated by *O. ribifloris* in southeastern United States contained fewer seeds than fruits pollinated by honey bees (Sampson and Cane 2000).

Sampson et al. (2009) did not recommend *O. lignaria* as a pollinator of blueberry, because even if *O. lignaria* can use its pollen and produce viable progeny when confined on blueberries, this bee has a strong preference for tree crops. This is supported by many other reports that indicate that most of the pollen found in *Osmia* nests located in agricultural environments belong to tree-crops, especially rosaceous trees (Vicens and Bosch 2000a, Monzón et al. 2004, Bosch et al. 2006, Sheffield, Westby, Smith, et al. 2008). Also, even if the foraging range is usually between 50-150 m from the nest (Bosch and Kemp 1999, Dogterom 1999), the possible flight range is close to 600 m (Zurbuchen et al. 2010), highlighting the importance of considering the location of the nest in the field.

There is growing interest in the use of alternative pollinators among growers who are experiencing rising costs for honey bee rentals, and increased variability in spring weather. Given this, it is important to explore pollen use by *O. lignaria* in field experiments in large areas of blueberry production to determine whether this bee can be used as an alternative pollinator of this crop, or whether the promising results described above are relevant only to those specific environments. To address this question, the objectives of this study were 1) to determine whether blueberry is a main pollen source for *O. lignaria* in a commercial field in Michigan through the bloom season, 2) to determine whether the location of the nest in the field influences the pollen source used by *O. lignaria*, and 3) to determine the plant species collected by *Osmia* in this setting.

METHODS

Study site

This experiment was carried out at the Blueberry Valley farm in Pullman, Michigan (USA) (42°28'40.42"N 86° 1'38.50"W). The farm has an area of approximately 79 ha, with 63 ha dedicated to highbush blueberry (*V. corymbosum*). The varieties grown and the percentage of area they occupy in the field were Jersey (33.1%), Bluecrop (24.8%), Nelson (14.2%), Elliott (14%), Rubel (4.4%), Northland (3.6%), Blue Jay (3%), Aurora (2.3%) and Blue Gold (0.6%). The farm uses conventional management practices, including insecticide and fungicide applications. During the bloom period insecticide applications are reduced and only methoxyfenozide was applied during this study, but fungicide applications continued, including chlorothalonil, captan and metconazole. Periodic mowing approximately every two weeks in and around the field was performed by the farmers. However, weeds and wild plants grow in the drainage channels around the edges of the field. The field is mainly surrounded by deciduous forest (Figure 4.1).



Figure 4.1. Blueberry Valley farm in Pullman Michigan outlined in red, and the locations (IN or OUT) where *Osmia* bee shelters were located.

A total of 16 locations in the field were selected for *Osmia* nests. Eight locations were positioned at the east or west border of the farm (OUT location), and the remaining eight locations were inside the farm (IN location) (Fig. 4.1). The inside locations were at least 200 m away from the border of the field, given that the foraging rang of *O. lignaria* is estimated to be between 50-150 m (Bosch and Kemp 1999, Dogterom 1999, Mader et al. 2010). Each location was a minimum of 100 m away from each other. When logistically feasible, I selected locations as far as possible (>50 m) from commercial honey bee hives placed within the field.

Experimental setup

Nesting shelters consisted of pine plywood boxes (26 x 28 x 28 cm) with an open face. Inside each box, I placed 25 bamboo stems and 25 natural reeds approximately 15 cm long with an inside diameter of 7-10 mm (Fig. 4.2). Boxes were held 1.5 m above ground by a metal post, and the open face of the box was covered with chicken wire to exclude birds and rodents. Shelters at the border of the field were placed facing into the field (east or west), and shelters in the field were placed with the opening parallel to the orientation of the plant rows (Fig. 4.2B). Shelters remained in the field between May 10 and June 17, 2016.

An emergence box containing 40 female and 60 male loose cocoons (at least 73% *O. lignaria,* with the remaining proportion being *O. cornifrons*) were placed in each shelter. The emergence box consisted of a cardboard box of 9.3 x 9.3 x 4.3 cm, with a hole on one face to allow bees to go out of the box after emergence. Cocoons were gathered from trap nests located at Michigan State University (MSU) campus in East Lansing, MI, in September 2015. Nests were opened and cocoons were extracted and sorted by sex based on size since female cocoons are bigger (≈ 11 mm) than male cocoons (≈ 6 m) (Torchio and Tepedino 1980). Cocoons were stored in an environmental chamber at 4°C from November 2015 until their release. A subsample of 50 cocoons was taken from each batch and emerged in the lab by holding them at laboratory conditions ($\approx 25^{\circ}$ C) to determine the proportion of each species.



Figure 4.2. Nest shelter located at A) the OUT location at the edge of the field and B) at the IN location in the blueberry field.

Pollen samples from nests

Shelters were inspected at least once per week during the study period to record the number of completed nests and to observe nesting bees. I observed each shelter for 15 min, identifying the species actively nesting there. On June 8, 2016, when blueberry bloom was over, nests with a mud plug at the entrance were brought back to the laboratory and kept in cold storage (-23°C) until processing. All nests were opened and pollen from individual brood cells was collected. I also recorded the total number of nests in each location and the number of cells per nest.

Pollen sampling from plants

Once per week after placement of the boxes and until the nest were collected, I sampled plants blooming around each shelter. Two transects of 100 x 3 m were established at each location, where I recorded presence of flowering plants. At the OUT locations, transects were done in north and south direction. At the IN locations, transects followed the orientation of the blueberry rows. The first time each plant was observed, a reference specimen was collected and pressed for further identification using Petrides (1972), Newcomb (1977), Peterson and McKenny (1996). Additionally, several flower buds were collected from each plant for pollen extraction. If flower buds were not available, open flowers were collected. Flowers and flower buds were stored in 70% ethanol until processing. Pollen was extracted by macerating the anthers into a container with ethanol, to later centrifuge the solution for 5 min at 3000 rpm. This pollen was processed by acetolysis (see below) and used as a reference library for identifying pollen collected from bee nests.

Pollen processing

All pollen samples were processed by acetolysis, following the protocol of Louveaux et al. (1978). Briefly, pollen was treated with an acetic anhydride acid and sulfuric acid solution (9:1 ratio), and then subjected to washes of 70% ethanol and then distilled water to dissolve tissues and remove lipids and debris. This protocol makes the diagnostic characteristics of the pollen grains more visible (Jones 2014). Acetolyzed samples from each brood cell were set up on microscope slides. For each slide, I counted and identified all pollen grains to the lowest taxonomic level possible in 15 fields of view at a magnification of 400x. Each field of view had a diameter of 450 µm. Pollen identification was done with the assistance of the reference pollen library from herbaceous and woody plants collected at the field site.

Data analysis

Only the data from *O. lignaria* nesting in shelters were included in the analysis. The proportion of each pollen type in each brood cell was calculated based on the number of grains. Shapiro-Wilk tests and diagnostic graphs (qq plots and residual plots) were used to assess the normality, and Bartlett's test was used to assess variance homogeneity between the locations (Shanks and Hutton 1976). In order to meet the normality and equal variance assumptions, I used an arcsine transformation on the data for the parametric analysis.

To determine which pollen type was more abundant in each location, the proportion of each pollen type was compared within each group by an analysis of variance (ANOVA) and a Tukey's test (p<0.05). To evaluate the effect of location (IN or OUT) on the percentage of each pollen species, and to compare the proportion of blueberry pollen between cells within each location group, as well as the number of nests and cells per nest obtained between locations, I

used the non-parametric Kruskal-Wallis Rank Sum Test (p<0.05). All data analyses were conducted in R version 3.1.1 (R Development Core Team, Vienna, Austria) using the "Agricolae" package.

RESULTS

Fifty different blooming plant species were recorded during the sampling (Table 4.1). A total of 47 species were identified at the OUT locations, with 7.9 ± 0.3 species/location (mean \pm SE). At the IN locations, I observed 37 species and 6.5 ± 0.3 species/location. Sixty-eight percent of the species were common to both location. The number of *O. lignaria* nests built at IN and OUT locations (4.5 ± 0.4 and 4.2 ± 0.3 nest/location respectively) was similar, with no significant differences ($X^2_1 = 0.228$, p = 0.63), as well as the number of cells build per nest (IN: 5.5 ± 0.2 cells/nest; OUT: 5.6 ± 0.1 cells/nest) ($X^2_1 = 0.125$, p = 0.72).

Table 4.1. Blooming plants present in the blueberry field during the period *Osmia lignaria* was active (May 10 – June 17, 2016), and their presence in the field interior (IN) or exterior (OUT).

Family	Species	Common name	Location	
Asteraceae	Taraxacum officinale	Common dandelion	Both	
	Hieracium pratense	Field hawkweed	Both	
	Senecio vulgaris	Common groundsel	IN	
	Erigeron annuus	Daisy fleabane	Both	
	Senecio jacobaea	Tansy ragwort	OUT	
	Achillea millefolium	Common yarrow	Both	

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Asteraceae	Chrysanthemum leucanthemum	Oxeye daisy	OUT
	Hieracium aurantiacum	Orange hawkweed	Both
	Hypochaeris radicata	Cat's ear	Both
Boraginaceae	Borago officinalis	Borage	IN
Brassicaceae	Barbarea vulgaris	Winter cress	Both
	Brassica rapa	Field mustard	Both
	Nasturtium officinale	Watercress	OUT
	<i>Rorippa</i> sp.	Yellowcresses	Both
	Sisymbrium sp.	Hedge mustard	IN
	Thlaspi arvense	Pennycress	Both
Caprifoliaceae	Lonicera tatarica	Tartarian honeysuckle	OUT
	Viburnum recognitum	Arrowwood	Both
Caryophyllaceae	Stellaria media	Common chickweed	Both
	Silene alba	White campion	Both
Cornaceae	Cornus florida	Flowering dogwood	OUT
	Cornus racemosa	Gray dogwood	Both
Cyperaceae	Carex sp.		Both
Ericaseae	Vaccinium corymbosum	Highbush blueberry	Both
Fabaceae	Trifolium repens	White clover	Both
	Trifolium pratense	Red clover	Both
	Medicago lupulina	Black medick	Both
	Vicia cracca	Cow vetch	OUT

Table 4.1 (cont'd)

Table 4.1 (cont'd)

Geraniaceae	Erodium cicutarium	Redstem filaree	OUT
Iridaceae	Iris versicolor	Blue flag iris	Both
	Sisyrinchium montanum	Blue eyed grass	Both
Lamiaceae	Lamium amplexicaule	Henbit	Both
Oxalidaceae	Oxalis stricta	Common wood sorrel	Both
Portulacaceae	Claytonia virginica	Spring beauty	Both
Ranunculaceae	Ranunculus ophioglossifolius	Spearwort	Both
	Ranunculus hispidus	Swamp buttercup	OUT
Rosaceae	Fragaria virginiana	Wild strawberry	Both
	Potentilla simplex	Oldfield cinquefoil	Both
	Prunus serotine	Black cherry	OUT
	Rubus sp.	Bramble	Both
	Rubus allegheniensis	Raspberry	OUT
Rubiaceae	Galium asprellum	Rough bedstraw	Both
Salicaceae	Salix pentandra	Laurel willow	Both
Scrophulariaceae	Nuttallanthus canadensis	Blue toadflax	Both
Solanaceae	Solanum dulcamara	Deadly nightshade	OUT
Violaceae Viola kitaibeliana		Field pansy	Both
	Viola sororia	Common blue violet	Both
	Viola lanceolata	Lance leaved violet	Both
	Unidentified bush 1 (P17)		OUT
	Unidentified tree 2 (P21)		OUT

There were 23 nests included in the pollen analysis, nine nests (with 83 cells total) from the IN location, and 14 nests (with 105 cells total) from the OUT locations. Overall, 81.8% of cells contained blueberry pollen, but only in 2.3% of the cells the percentage of blueberry pollen was higher than 50%, and only in 1.5% the contribution was higher than 90% (Fig. 4.3). The percentage of blueberry pollen in cells was not different between locations (IN: 4.9 ± 1.0 %; OUT: 5.5 ± 1.1 %) ($X^2_1 = 0.450$, p = 0.502) (Fig. 4.3). Nine other pollen types were identified in cell provisions (Fig. 4.4), including black cherry (*Prunus serotina*, Rosaceae) and white clover (*Trifolium repens*, Fabaceae). At the OUT locations, percentage of pollen from white clover and *Rubus* spp. were 6.4 and 3.4 % higher than at the IN locations respectively (all p < 0.05), while black cherry showed a 12.4% highest abundance at the IN locations ($X^2_1 = 6.96$, p = 0.008) (Fig. 4.4). The percentage of blueberry pollen did not change among cells of the same nest regardless of the locations, and therefore the timing of their construction (all p > 0.05) (Fig. 4.5).



Figure 4.3. Percentage of blueberry pollen in *O. lignaria* nests located inside (IN) or at the edge (OUT) of a blueberry farm in West Michigan.



Figure 4.4. Mean (\pm SE) percentage of the most common pollen found in the brood cells of *O*. *lignaria* nesting inside (IN) or at the edge (OUT) of blueberry fields in west Michigan. The * represent statistical differences between positions (IN and OUT) (p < 0.05).

In both locations the most abundant pollen belonged to black cherry and white clover, together accounting for 84% of the pollen provision at the IN locations and 79% at the OUT locations. The percentage of blueberry pollen was significantly lower than these two plants in both location (all p < 0.05) (Table. 4.2). Other pollen types present in the brood cells in percentages lower than 0.1% include *Rubus allegheniensis* (Rocaceae), *Silene alba* (Caryophyllaceae) and an unidentified pollen type.



Figure 4.5. Percentage of blueberry pollen in *Osmia lignaria* cell provisions collected at the interior (IN) and exterior (OUT) of the field as a function of their position in the tubes, equating to their timing of completion of the cells.

Table 4.2. Mean percentage (\pm SE) of total pollen in *O. lignaria* brood cells nesting inside (IN) or at the edge (OUT) of blueberry fields in west Michigan. Within each location, means with the same letter are not significantly different, p > 0.05.

	Prunus	Prunus Trifolium		Vaccinium	
	serotina	repens	pentandra	corymbosum	
IN	54.1 ± 1.7 a	30.7 ± 1.9 b	6.8 ± 3.7 c	6.3 ± 4.0 c	
OUT	41.7 ± 0.7 a	37.1 ± 2.2 a	10.6 ± 3.2 a	4.7 ± 3.5 a	

DISCUSSION

In this study I found that *O. lignaria* nest cells contain pollen from blueberry plants regardless of whether they are nesting inside or at the edge of the field. However, blueberry is not one of the main pollen sources used by these bees, representing a low proportion of total collected pollen (Fig. 4.4). Additionally, the proportion of blueberry pollen did not change among cells in the nest, suggesting that the use of blueberry pollen was relatively consistent over time (Fig. 4.5), independent of the volume of crop bloom open in the field.

Osmia species, including O. lignaria, are able to gather blueberry pollen despite of its bell-shaped corolla and poricidal anthers that can restrict the access to many other pollinators (Stubbs and Drummond 1997, Dogterom 1999, Wilkaniee and Giejdasz 2003, West and McCutcheon 2009). I also found a few cells that contained more than 90% blueberry pollen, which further indicates that access to the flowers is not a limitation for these bees. However, O. lignaria, as well as other Osmia species, show strong floral preferences, mainly for plants of the family Rosaceae (Williams and Tepedino 2003, Sampson et al. 2009, Mader et al. 2010). When bees are released during the bloom period of tree crops such as apples, cherries or pears, most of the pollen found in their nests (up to 95%) corresponds to pollen from the main crop (Vicens and Bosch 2000b, Bosch et al. 2006, Sheffield, Westby, Smith, et al. 2008, Kraemer et al. 2014). Even when released in orchards, however, not-target plants can be more attractive than the target crop. Kraemer et al. (2014), reported that in some apple orchards the most abundant pollen in O. lignaria brood cells was Eastern redbud (Cercis canadensis: Fabaceae), and even in natural habitats mason bees also show strong floral preferences (Radmacher and Strohm 2010). In this case I found that the most abundant pollen in cell provisions belonged to back cherry and white

clover, both plants in families that have been reported to be highly attractive to *Osmia* (Rosaceae and Fabaceae).

In general, *O. lignaria* tend to collect resources that are close to their nests, but this can be affected by plant density and plant species (Williams and Tepedino 2003). Black cherry is a dominant native species of tree in the woods of this region and is commonly found in woodlands that were cleared to create fields for blueberry fields. Black cherry was present only at the edge of the fields, so females nesting in the field had to fly at least 200 m to collect this pollen. The foraging range of *O. lingaria* is between 50-150 m (Bosch and Kemp 1999, Dogterom 1999), but they can fly up to 600 m (Zurbuchen et al. 2010), so it is perhaps not surprising that bees left the field to collect pollen from plants that are highly attractive to them. Additionally, blueberry pollen has a low protein content (13.9%) (Somerville 2001), suggesting that it is not sufficiently nutritious for *Osmia* to use this as a dominant pollen to feed their larvae, a fact that has been also observed in honey bees (Topitzhofer 2014).

Whether *Osmia* bees are effective pollinators may have more to do with their interaction with the flowers than whether their pollen provisions contain a high proportion of blueberry pollen. The pollination efficiency of some bees on blueberries is variety-dependent. For example, *O. ribifloris* effectively pollinates Tifblue and Climax rabbiteye blueberry, but not Premier, while *A. mellifera* is an effective pollinator of Premier and Climax, but not Tifblue (Sampson and Cane 2000). Visitation rates of *O. ribifloris* decrease for varieties with narrow corollas (Courcelles et al. 2013), while varieties with long pistils are more efficiently pollinated because pollen transfer is made easier for the bees (Sampson et al. 2013). It is possible that the small peaks observed in blueberry pollen collected during the nesting period of *O. lignaria* (Fig 4.5), correspond to the blooming period of different blueberry cultivars with differences in their floral

morphology, that could make easier for bees to harvest its pollen. Therefore, it would be necessary to evaluate whether pollination efficiency and flower preferences change for different varieties of highbush blueberry, through direct observations of the bees in multi-variety plantings.

The presence of honey bees in high densities is also a factor that can negatively affect visitation rate of wild bees (Lindström et al. 2016). Bumblebee visits to wild plants increase as distance from honey bees hives increase (Thomson 2006), and they can also shift between plant species when honey bee density increases (Walther-Hellwig et al. 2006). *Osmia* bees can be affected by a high density of honey bees, decreasing visitation rate to blueberries and reducing the number of cells built per nest by *O. bicornis* in a cage experiment (Hudewenz and Klein 2015). In our study site, honeybee hives were placed into the blueberry field to ensure good pollination levels, and it is possible that the high rate of honey bees visiting blueberry flowers could have negatively influenced visitation rate by *O. lignaria*, making them more likely to visit other plants.

Another farm practice that can affect bees is the applications of pesticides (Johnson 2015). At the Blueberry Valley site, they restrict the applications of insecticides during bloom only to pesticides with high safety for bees, but there are applications of fungicides during that period. No lethal effects of fungicides have been reported for *O. lignaria*, but after applications of iprodione, propiconazole, benomyl, and captan, bees can remain inactive for a few hours before resuming activity (Ladurner et al. 2008). Artz and Pitts-Singer (2015) found that iprodione, pyraclostrobin and the adjuvant N-90 disrupt the nest recognition abilities of *O. lignaria* females. Though the fungicides (chlorothalonil, captan and metconazole) and insecticide (methoxyfenozide) applied in our experimental site did not seem to discourage bees from nesting

at the IN locations, they could have caused a repellent effect on *O. lignaria*, making blueberry flowers less attractive to bees. This could be another possible explanation for the variation observed on the percentage of blueberry pollen in different cells, due to that application of pesticides could induce bees to not visit blueberry flowers for a short period. However, it is difficult to estimate the exact day a specific cell was build, so it is difficult to establish a relationship between blueberry pollen use and pesticides applications. Furthermore, this was not measured directly, and further studies of pollinator-pesticide interactions in this system would be important for better understanding the importance of these potential interactions.

In Michigan, O. lignaria fly between April and May, so their nesting period does not completely coincide with blueberry bloom, which typically happens during May (Garcia-Salazar 2002). The possibility of adjusting the timing of O. lignaria emergence is one of the most appealing characteristics of this species, because it allows for the ability to release a large number of individuals to cover the bloom period of the crop. However, releasing bees out of their normal flight period also potentially exposes them to plants different from those they would normally encounter, resulting in females bringing atypical pollen back to the nest. Pollen of different plant species differ in their nutritional value and digestibility (Roulston and Cane 2000), and they can also differentially influence larval growth and development in several bee species, including O. lignaria (Levin and Haydak 1957, Tasei and Aupinel 2008). The use of non-host species can have negative effects on larvae health, as in the case of some populations of O. cornuta, that when reared with Ranunculus acris (Ranunculaceae) pollen, a non-host species for this bee, had higher larval mortality (Haider et al. 2013). This negative effect of non-host pollen can be due to physiological limitations related to pollen digestion, including lack of essential nutrients or the presence of toxins in the pollen (Williams 2003, Sedivy et al. 2008). This

highlights the importance of considering several factors when releasing *O. lignaria* in a time period different to their natural emergence time and in locations where the species does not naturally occur (Pitts-Singer et al. 2014).

Plant diversity in and around farms has been proposed and evaluated as a method to increase pollinator diversity in agroecosystems (Potts, Biesmeijer, et al. 2010). By providing alternative pollen and nectar sources, bees can establish before the main crop blooms, and continue providing resources after it is over, to allow bees to have the highest reproductive success rate (Stubbs et al. 1992, Sedivy and Dorn 2014, Werling et al. 2014). However, co-blooming alternative foraging plants should not be more attractive to bees than the crop of interest, otherwise the objective of pollinating the crop may not be accomplished (Bosch and Kemp 2002, Mader et al. 2010). Considering that *O. lignaria* preferred to forage on alternative plants in this system, this species may not be the best option to use as an alternative pollinator for blueberry, and there should be future exploration for additional bee species that may have greater fidelity to blueberry.

With the results obtained in this experiment I can conclude that in field conditions *O*. *lignaria* does not visit blueberries as a main source of pollen, and therefore their contribution to pollination of this crop is probably low. The fact that *O*. *lignaria* does not have a high level of visitation to blueberries, even when there is a large amount of pollen available during bloom, suggest that different factors including floral preferences, competition with honey bees, and application of fungicides during bloom may limit its ability to provide high levels of pollination.

Even though *O. lignaria* does not appear to be a promising pollinator of blueberry, there are many other *Osmia* species that have been observed visiting blueberry fields, including *O. atriventris, O. pumila* and *O. bucephala* (Stubbs et al. 1992, Stubbs and Drummond 1997,

Sampson et al. 2009b, Tuell et al. 2009; J. Gibbs unpublished). Although their relative abundance can be low (Ratti 2006, Bushmann and Drummond 2015, Scott et al. 2016), it could be increased by providing suitable nesting substrates (Torchio 1982a, Mader et al. 2010, MacIvor 2016). Several studies show that increasing diversity and abundance of bees in agricultural areas can increase yield stability, resilience, and decrease dependence and pressure on honey bees (Garibaldi et al. 2013, 2016, Senapathi et al. 2015, Blitzer et al. 2016). Given this, practices that help to establish and increase wild pollinator populations around farms can have a positive effect on yield, as shown by Gibbs et al. (2016), who found that in British Columbia blueberry farms, wild bee abundance is related to the proportion of semi-natural habitat around the field, and the pollination service increased with abundance of wild bumble bees.

In this study I found that blueberry pollen can be found in *O. lignaria* nest provisions, but at low levels in comparison with other pollen types. Additionally, the proportion of blueberry pollen does not change during the nesting period or depending on the location of the nest (in the field or at the border of the field). These finding are important given the growing interest of using alternative pollinators for crops, and in this case show that *O. lignaria* would not be the best option to pollinate blueberries. Future studies that aim to use *Osmia* as blueberry pollinators could focus on other *Osmia* species that naturally visit blueberries and how to increase their visitation rate. Plants with high pollen contribution identified in this study could be used as pollen sources for *O. lignaria* in non-agricultural setting, like urban environments, where they can be important pollen sources for several bee species, including *Osmia*.

CHAPTER 5. FUTURE DIRECTIONS

Mason bees (*Osmia* spp.) are a promising group of alternative pollinators for rosaceous fruitcrops. Although reports about mason bees biology and behavior started early in 1925, and several studies about their nesting preferences and potential for crop pollinations were published in the 1980s, only in recent years has there been a concerted effort to have these pollinators readily available for commercial orchards. Indeed there are now commercial suppliers of these bees, supplying the residential and farm markets for *Osmia* bees. However, the extensive use of mason bees in orchards requires us to have sufficient knowledge of their biology and behavior to be able to ensure an adequate supply of bees to provide for crop pollinations. While the results obtained in the present thesis contributes to the body of knowledge that will help to improve management of *Osmia* bees as alternative pollinators, there are still issues and questions. Next I will present future directions and further questions generated from my results that still need to be addressed.

In the shelter/substrate experiments I found that a higher percentage of cavities are used by *O. cornifrons* than *O. lignaria,* and a larger number of female offspring is produced when using wooden shelters and reeds. So this combination should be tested in commercial settings to establish the degree of success in the environments that bees are being used for commercial pollination. However, this combinations of shelter and substrate materials is more expensive than plastic shelter and cardboard tubes, so there is also a need for economic analyses of whether the investment in more expensive materials is balanced by the increase in pollination service or number of offspring produced that could be use in the next growing season.

The attractant spray tested in this research proved to be effective at increasing the number of cavities used by *O. cornifrons,* so further research could focus on testing different

concentrations and the degradation time of the active ingredient, so it can be established whether and when reapplication is needed. Additionally, the attractant should be tested in a broader range of species. Another point that should be addressed is that even when the attractant was originally developed from *O. lignaria* cocoons, in this experiment I observed a low response of this species to the attractant. These results could be influenced by the high dispersal rate that is commonly reported for *O. lignaria*, so a larger number of females should be used in future experiments to be able identify possible effects of the attractant on this species. This also highlights the importance of developing rearing and releasing methodologies that could decrease dispersal of *Osmia* bees in the field.

Research that focuses on the ecology of species that have the potential to be used as commercial pollinators is also an important topic. Given that *O. cornifrons* is a successful introduced species that has spread widely through the eastern US, possible negative effects on native species due to competition for floral or nesting resources should be conducted before introducing this species in areas where it is still not present. Studies on the effects of trapping of wild populations of *Osmia* with the aim of extracting individuals to be used to supplement artificial rearing, or for research purposes, would be important to establish the degree in which that practice can affect natural populations.

Regarding soil used by *Osmia*, this is the first study that has addressed detailed particle size analysis of the soil collected by bees. I found that bees can used a wide range of soil textures, unlike the information provided in previous publications, and that there are differences in the texture of the soil collected by both species. I also found variation in the consistency of individual females *Osmia* bees for collecting soil, with different soil types being found within a single partition, among partitions, and among nests. This generates a significant set of new

questions regarding soil used by mason bees, including; what soil texture would females prefer if a wide range of soil textures is offered? Are females able to use soil of least preferred texture if other options are not available? Does the soil texture of the partitions influence its effectiveness at preventing the entrance of parasites/predators into the nest? Do different populations or subspecies prefer soil of different textures? What other parameters (like organic matter content, bulk density, moisture content) are important for bees when selecting soil sources? How far can females fly to collect soil of their preferred characteristics? These and several more questions are of interest not only to better understand the ecology of mason bees, but also to be able to determine whether a particular region offers soil suitable for bees or whether it is necessary to provide additional soil sources of the adequate characteristics.

For the blueberry pollen use experiment, I found that even when nests are located in a big blueberry field more than 200 m away from the border of the field, *O. lignaria* females prefer to collect pollen from alternative plants that bloom in or around the field. These results support other studies that suggest that this species is not a suitable pollinator for blueberries given that they do not visit the target flowers if other floral options are available. These results are important given that recently more attention has been paid to alternative pollinators, and growers could mistakenly interpret that *O. lignaria* is an effective pollinator of blueberries in the field However, *O. cornifrons* still remains to be tested in the field to determine whether they will constantly visit blueberries. Additionally, other *Osmia* species have been reported to naturally visit blueberry flowers, like *O. atriventris, O. pumila* and *O. bucephala*, so they can be asses for their effectiveness and potential as blueberry pollinators. Studies about their nesting preferences would be helpful even if the species are not suitable for mass rearing, given that nesting substrates can be provided around fields to increase natural occurring populations of these bees

Even if substantial progress has been made on mason bee rearing and management for orchard pollination in recent years, is clear that we still have to solve several questions that will allow us to have a sustainable and reliable system to rear and manage *Osmia* bees for crop pollination, reducing dependence and pressure on honey bees. In that sense, the present work makes a substantial contribution. However, we would also have to implement management strategies that are compatible with the use these pollinators, and recognize that it will not be possible to have commercial pollinators for every crop, and that in many cases integrated management of the field is necessary to ensure a long-term pollination service that does not only rely on human inputs.

APPENDIX

APPENDIX A

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2017-06

Author and Title of thesis: Mario Simon Pinilla Gallego. "Nest, soil and pollen preferences of mason bees (Hymenoptera: Megachilidae: *Osmia*) in Southwest Michigan farms".

Museum where deposited: Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Sex	Quantity	Preservation
Hymenoptera	Osmia lignaria	Adult	Male	10	Pinned
Hymenoptera	Osmia lignaria	Adult	Female	10	Pinned
Hymenoptera	Osmia cornifrons	Adult	Male	10	Pinned
Hymenoptera	Osmia cornifrons	Adult	Female	10	Pinned

Table A. List of voucher specimens

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LITERATURE CITED

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