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The Use of Trap-Nests to Manage Carpenter Bees (Hymenoptera: Apidae: Xylocopini), Pollinators of Passion Fruit (Passifloraceae: *Passiflora edulis* f. *flavicarpa*)

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ABSTRACT Carpenter bees are the main pollinators of passion fruit, a crop classified as vulnerable to pollinator decline because it is strictly self-incompatible. We investigated cost-effective management strategies to increase the presence of female carpenter bees in passion fruit orchards by using trap-nests in bee shelters. Transfers of nests containing females of *X. frontalis* and *X. grisescens* between different sites were significantly more successful when the nests contained brood cells. Supplying a bee shelter with a combination of suitably sized empty bamboo stalks and active nests of carpenter bees can increase the population of actively nesting bees by >200% during the course of 23 mo, as a consequence of the emergence of brood from the introduced nests and the attraction of bees from the surroundings. In conclusion, our methods lead to improved success of introducing, increasing and maintaining carpenter bees populations for the pollination of passion fruit crops.

KEY WORDS solitary bee, bee management, nest reuse, pollination

Solitary bees that nests in pre-existing cavities are good candidates for management for sustainable pollination in agroecosystems. In the United States and Europe, *Megachile rotundata* F. (Hymenoptera: Megachilidae) is used in the alfalfa (*Medicago sativa* L.) and blueberry pollination (Stubbs et al. 1994, Pitts-Singer and Cane 2011) and the orchard bees, *Osmia* spp. (Hymenoptera: Megachilidae), are also successfully managed in fruit-growing areas (Bosch and Kemp 2000, 2002; Vicens and Bosch 2000; Maccagnani et al. 2003).

Solitary or facultative social bees of the genus *Xylocopa* Latreille (carpenter bees) (Hymenoptera: Apidae: Xylocopini) are considered effective pollinators of a range of native and cultivated plant species (Corbet and Willmer 1980, Oliveira and Gibbs 2000, Carvalho and Oliveira 2003) because they are generalist foragers and demonstrate some floral constancy (Frankie and Vinson 1977, Gerling et al. 1989).

Carpenter bees are large and robust with a length up to 4.5 cm (Michener 2007). The genus contains >700 species worldwide, and 50 species are known to occur in Brazil (Silveira et al. 2002, Michener 2007). Most of the species excavate their nests in dry plant tissue, such as trees or dead trunks (Camillo and Garófalo 1982, Camillo et al. 1986, Chaves-Alves et al. 2011) and hollow stems (Ramalho et al. 2004) but they can also occupy pre-existing cavities such as artificial nests made of bamboo canes (Camillo 2003, Marchi and Melo 2010, Pereira and Garófalo 2010, Chaves-Alves et al. 2011).

In southeastern Brazil, *Xylocopa* (*Neoxylocopa*) *frontalis* (Olivier, 1789) and *X.* (*Neoxylocopa*) *grisescens* Lepeletier, 1841 are the main pollinators of passion fruit (*Passiflora edulis* f. *flavicarpa* Degener, Passifloraceae) (Yamamoto et al. 2012), a crop classified as vulnerable to pollinator decline (Ghazoul 2005) because it is a strictly self-incompatible fruit crop (Rêgo et al. 2000).

Brazil is the main producer of passion fruit with an estimated annual production of 380,000 tons (Araújo et al. 2005). To improve production, local farmers use hand pollination techniques that increase production costs up to 12% (Agrianual 2011). An alternative to this expensive technique is the management of carpenter bees in crop areas, which can result in an increase of both fruit set and fruit quality (Camillo 2003).

Carpenter bees are considered suitable for management in crop areas because they will take up trap-nests (Oliveira-Filho and Freitas 2003, Pereira and Garófalo 2010), which can be moved and allow local population increases. Studies carried out in Brazil have demonstrated that these species use trap-nests made of bamboo canes and wood trunks with suitable texture for digging (Camillo and Garófalo 1982, 1989; Camillo 2003; Chaves-Alves et al. 2011). They also use wooden boards in nests boxes based on Langstroth hive, which allows both management and behavioral studies of these species (Freitas and Oliveira-Filho 2001, Oliveira-Filho and Freitas 2003). Among the artificial nesting substrates, trap-nests made of bamboo cane

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are the most viable alternative for carpenter bee management because they are affordable, relatively easy to provide and replace, and well accepted by the bees (Chaves-Alves et al. 2011).

Given that there is both a need and a potential to enhance populations of *Xylocopa* spp. in passion fruit orchards, we assessed the effectiveness of specific management procedures of *X. frontalis* and *X. grisescens.* Specifically, we measured: 1) the effectiveness in transferring established nests of *X. frontalis* and *X. grisescens* between areas, 2) whether such transfers lead to an enhanced density of bees nesting in the area, and 3) the frequency of philopatry and dispersal by emerging females.

Materials and Methods

Study Site. The study was conducted at Água Limpa Experimental Farm (19° 05′48″ S, 48° 21′05″ W), which belongs to the Federal University of Uberlândia, Uberlândia, Minas Gerais, from March 2008 to January 2010. The climate is tropical with two defined seasons, a rainy summer (October to March) and a dry winter (April to September) (Rosa et al. 1991).

Água Limpa Experimental Farm is ≈60 ha in size, and includes remnants of cerrado, cerrado sensu stricto, palms and gallery forest. The area also contains ≈17 ha of mainly fruit crops, including mango (*Mangifera indica* L.), West Indian cherry (*Malpighia emarginata* DC.), passion fruit (*Passiflora edulis* Sims), pineapple [*Ananas comosus* (L.) Merr.], guava (*Psidium guajava* L.), tamarind (*Tamarindus indica* L.) , and several other crops (Neto 2008).

Trap-Nests. Two experimental bee shelters of 2.5 m high and 1.5 m long were built from wood rafters and plastic canvas covers, and situated at ≈200-m distance from each. They provided shelter for the trap-nests and allowed us to monitor the dynamics of their occupation by *X. frontalis* and *X. grisescens.* Each bee shelter was supplied with 192 trap-nests made of bamboo canes, closed at one end by the node, with a length of ≈25 cm and an inner diameter between 1.41 and 2.40 cm. The diameter range of the trap-nests was based on the minimum and maximum diameters of natural nests of *X. frontalis* and *X. grisescens* (Camillo and Garófalo 1982, Camillo et al. 1986). In the bee shelter, the trap-nests were placed in hollow bricks that were arranged horizontally on shelves and functioned as support. Access by ants was prevented by applying Tanglefoot (Contech Enterprises, Victoria, BC, Canada) to the supports of bee shelters.

Nest Introduction. In total, 12 nests of *X. grisescens* and 17 nests of *X. frontalis* were transferred to the experimental shelters ("introduced nests"). These nests were removed from two other bee shelters (original shelters), at 12-km distance to the study site. These nests had been founded in trap-nests, and contained either both adult females and brood cells (1–4 cells, $n = 13$ nests) or solitary adult females only ($n = 16$ nests).

The nests were transferred during two periods in March 2008, during the rainy season ($n = 14$ nests) and in June 2008, during the dry period ($n = 15$ nests). Before transfer, the nests were monitored for nesting activity at the original bee shelters to allow selection of the nests according to brood presence or absence. The bees within the transferred nests and the females from the surrounding area that had initiated nest foundation in the shelters were marked individually with numbered tags by using the methods of Yamamoto (2009). This allowed monitoring the females with respect to nest switching, foundation of new nests, and nest reuse. This also allowed an assessment of the frequency of females that remained in the original nest after transfer.

The study site was visited twice a month to monitor the introduced nests and observe the occurrence of new foundations. During these visits, which took place from March 2008 to January 2010, between 1000 and 1400 hours, an otoscope was used to identify individual adult occupants by their numbered tags and record the presence of brood cells in the trap-nests.

During these inspections, we checked: 1) number of newly founded nests, identified as nests that had been recently founded, without brood cells, but in which the founder females had already started their foraging activities; 2) the number of active nests founded, i.e., nests previously founded and with adult females and brood cells; 3) number of active nests reused by emerging females, in the presence or absence of mother; and 4) the number of abandoned nests.

Data Analysis. Statistical analysis was performed using the statistical program Systat (2002) (Zar 1999). χ^2 test was performed to compare the effectiveness of nest transfer by species and by season and also to compare the number of nest foundation by season. To analyze the number of cells built in the nests where the females remained after the nest transfer and in those in which they abandoned, we used a Mann–Whitney test. The mean values are followed by standard errors.

Results

Effectivity of Transfers. The females remained in 54% (16 of 29) of the introduced nests. There was no significant difference between the species ($\chi^2 = 0.258$, $P > 0.05$), with females remaining in 50% (nine of 17) of the introduced nests containing *X. frontalis* and in 60% (seven of 12) of the nests containing *X. grisescens.*

After transfer, the nests in which the adult females remained contained on average higher numbers of brood cells (1.45 ± 1.5 cells, $n = 15$ nests) than the nests from which the adult females had disappeared (0.50 ± 1.02 cells, $n = 14$ nests; $U_{0.05(2)14,15} = 64$, $P = 0.04$). The females that abandoned their nests after transfer did not initiate nesting activity in any of the available substrates at bee shelters. Those that remained in their nest after transfer were observed until emergence and dispersal of their offspring.

The permanence of females after transfer was not influenced by season. The percentage of females that remained in their nests was 57% (nine of 15 introduced nests) during the rainy season and 50% (seven of 14

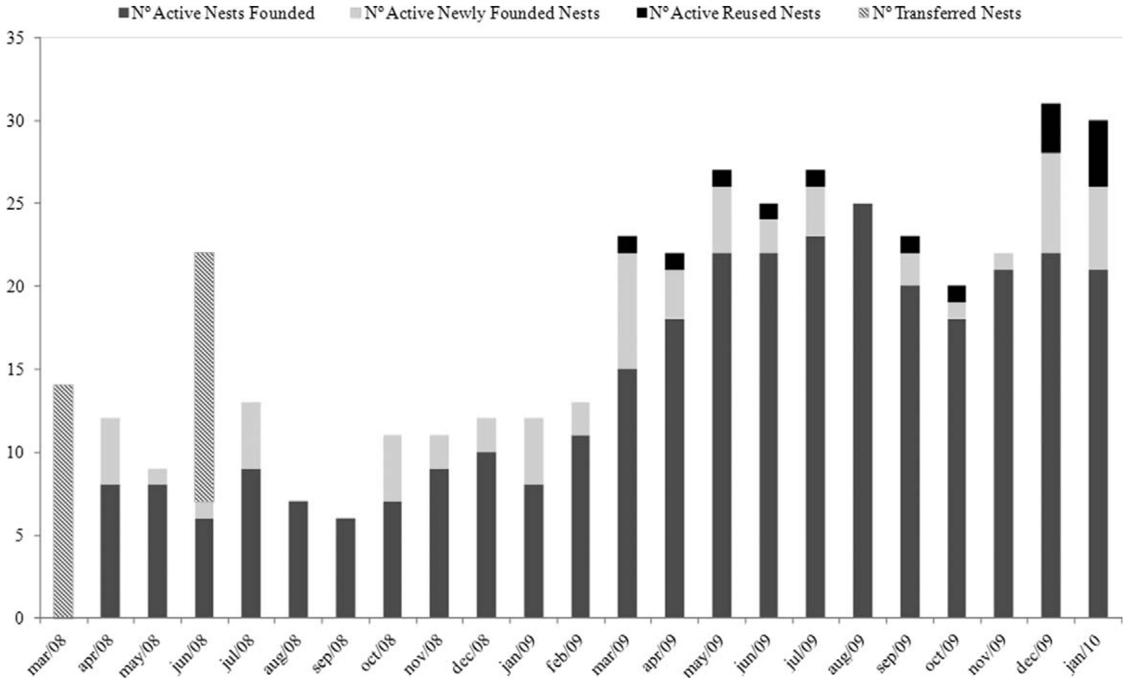

Fig. 1. Variation in the number of active nests of carpenter bees over the study period, from March of 2008 to January of 2010. Hatched: Number of nests transferred, gray: Number of active newly founded nests, black: Number of active reused nests, dark gray: Number of active nests founded.

introduced nests) in the dry season ($\chi^2 = 0.144$, $P > 0.05$).

Nest Founding and Reuse at Bee Shelters. At the experimental shelters, 58 new nests were founded and 14 nests were reused either after abandonment by the female or emergence of the brood during the study period, 79% ($n = 57$) by *X. frontalis* and 21% ($n = 15$) by *X. grisescens.* Most nest foundation and reuse occurred in the rainy season for both species (Fig. 1).

Of the females' offspring that emerged from the introduced nests or from nests founded in the experimental shelters during the study period, 132 were marked. Sixty of them remained in the study site, but only 36 females founded or reused nests. The other ones remained at maternal nests without brood cell production and dispersed after ≈30 d after emergence. In addition, 36 new nests were founded by females from the surrounding area that were attracted to trap-nests.

The average diameter of the cavity of occupied trap-nests was 19.7 ± 2.1 mm (range: 15.5–24.0 mm) for *X. frontalis* and 20.1 ± 2.0 mm (range: 17.1–23.1 mm) for *X. grisescens.* Thus, it seems that trap-nests with an inner diameter of <18 mm and >22 mm were not suitable. Of all trap-nests available in the study site, 21% became occupied. Taking into account only the nests substrate of suitable diameter, the percentage of occupancy was 45%, 34% for *X. frontalis* and 11% for *X. grisescens.*

The number of active nests founded and reused and newly founded nests fluctuated throughout the study period (Fig. 1). An increase of nests occurred by founding and reusing of new nests substrate and a decreased by abandonment of nests in development (with brood cells) or when females dispersed after emergence. Considering the number of introduced nests ($n = 29$) and the total number founded and reused during the study period ($n = 72$), the number of occupied nests increased by 248%.

The number of founded nests increased after the first year of study. Both *Xylocopa frontalis* and *X. grisescens* actively provisioned cells throughout the year and produced more than one generation per year. During the study period, a maximum of 30 active nests were observed simultaneously. The number of nest foundations was higher during the rainy season than during the dry season ($\chi^2 = 4.5$, $P < 0.05$; Fig. 1).

Regarding natural enemies, we observed only one nest of *X. frontalis* with brood cells parasitized by the cleptoparasitic *Cissites* sp. (Coleoptera, Meloidae).

Discussion

After initial introduction of a limited number of nests of *Xylocopa*, a large increase in the use of trap-nests made of bamboo canes was observed over the course of 23 mo. This demonstrates that providing empty trap-nests in combination with the introduction of a "seed population" of bees can be an effective, affordable way of establishing populations of *Xylocopa* bees in passion fruit orchards.

The population build up was not only caused by emerging brood from the seed population, but also by females that were attracted from the environment.

Compared with solid wood, the attractiveness of bamboo nesting substrate may be associated with a smaller nest-building effort, because the gallery is nearly ready (Maeta et al. 1996). Because carpenter bees generally do not show an affinity to substrate of specific plant taxa (Bernardino and Gaglianone 2008), it is likely that any substrate with suitable characteristics, such as wood density or length of gallery, is suitable for the management of carpenter bees (Gerling et al. 1989).

Transfer of active nests containing brood cells lead to a higher retention of introduced adult females. In this study females from nests with brood cells had a permanence success of over 80%, which was higher than obtained with the use of nests boxes based on Langstroth hives to increase the number of individuals of carpenter bees in a passion fruit orchard (Freitas and Oliveira-Filho 2001).

There are two possible explanations for the difference in permanence between females introduced with and without brood, which are not mutually exclusive. First, the nesting activity may improve the reorientation capacity of females at a new site after nest transfer. It is well known that solitary bees and wasps can be disoriented and abandon their nests after a nest transfer or a change at the landmark lay-out (Zeil 1993, Fauria and Campan 1998, Bosch and Kemp 2001). Studies of *Xylocopa* spp. nesting behavior show that females mark their nest and use olfactory cues and individual odors (Anzenberger 1986, Hefetz 1992), and it is possible that these markings become more pronounced with nest use and improve reorientation.

Second, in *Xylocopa* species, the presence of the mother throughout brood development is crucial for the protection of the brood against predators (Watmough 1974). Newly emerged carpenter bees remain in maternal nests for up to 30 d after eclosion before they reach physiological maturity. During this period they are fed by the mother through trophallaxis (Camillo and Garófalo 1989), and consume pollen supplies brought in by the mother (van der Blom and Velthuis 1988). Therefore, the investment already made by females in active nests combined with obligate maternal care for emerging brood, may provide additional motivation to remain with the brood after transfer of the nest to a different location. The importance of the presence of brood for remaining in the nest after a conspecific take-over of dominance has been demonstrated for *Xylocopa pubescens* (Spinola) (Hogendoorn and Leys 1993).

In addition to the successful transfer of nests that contained brood, an increase of >200% was observed in the number of nests at the shelters because of new foundations, both by females that did not originate from introduced nests and by philopatric behavior (i.e., offspring that emerged at bee shelters and founded nests at the same location) (Yanega 1990, Antonini et al. 2000) or reused maternal nest (Camillo 2003). The attraction of females from the surroundings might be related to the presence of active nests and/or because of aggregation pheromones (Roubik 1989) as reported for other *Xycolopa* species (Michener 1979, Camillo and Garófalo 1989) or by limitations in suitable nesting substrates and floral resources (Wcislo and Cane 1996, Viana et al. 2002). Both philopatry and attraction are likely to enhance the population sizes at shelters over time.

In the time frame of our study, the aggregation of carpenter bee nests in bee shelters did not increase the occurrence of brood parasitism. Similar to other studies (Bernardino and Gaglianone 2008, Pereira and Garófalo 2010) there was a low incidence of parasitism by beetles of the genus *Cissites* that reinforces the suitability of bee shelters for *X. frontalis* and *X. grisescens* management.

In the long run, maintenance of populations of carpenter bees in passion fruit orchards requires continued nest protection from ant raids (Gerling et al. 1989), a regular supply of fresh nesting materials of suitable sizes, and plantings of other crop species in the surrounding of the orchards to maintain continued food resources because passion fruit it is only a nectar source (Camillo 2003). When using bamboo cane, the maintenance of a supply of fresh nesting substrate is of particular importance. Depending on thickness of the wall, bamboo canes can be reused only up to four times (Gerling et al. 1983, Chaves-Alves 2009), and they allow only linear nests, and no further excavations.

Among the species studied, the nest numbers of *X. frontalis* increased more than those of *X. grisescens.* Because *X. frontalis* is one of the most abundant species, with a wide distribution and a very wide range of food resources and nesting substrates (Gerling et al. 1989, Silveira et al. 2002), it may be a more plastic and therefore potentially more suitable species for management as a crop pollinator.

In conclusion, our study shows that simple, cost-effective measures can enhance the abundance of *Xylocopa* nests in passion fruit orchards. Further research is needed to formulate advice to farmers concerning the supply of new nesting materials and the removal of nests that are past their use-by date. In addition, the year-round maintenance of carpenter bees in cropping areas that have depauperate vegetation requires further attention.

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