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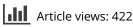
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# **ORIGINAL RESEARCH ARTICLE**

# Wild solitary bees and their use of bee hotels in southwest Spain

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There is an increasing interest in preserving and, if possible, increasing wild bee populations as evidenced by increasing investigations into providing supplemental nesting resources, commonly called bee hotels. The study presented here was carried out in 2017 and 2018 with two objectives: a) to understand the role that insect refuges could have on beneficial arthropod fauna, especially bees, and b) to evaluate different materials and which species used them. We present the preliminary results of three constructed refuges in Seville, Spain: Hymenoptera visited the refuges most frequently (88.7% of the visitors), of which the social wasps (*Polistes dominula* (Christ)) were most common, followed by bees. Bees were observed visiting bamboo canes, Arundo canes, drilled logs, and grooved boards. Drilled logs were the most used material (31.5 and 37.6% occupied in 2017 and 2018, respectively), followed by bamboo canes (14.1 and 17.4% of occupied in 2017 and 2018, respectively). For drilled logs, holes of 4.9–6.5 mm (2017) and 7.0–9.2 mm (2018) were preferred, whilst diameters of 2.6/2.7–4.9/5.0 mm (both 2017 and 2018) were preferred for bamboo canes. For grooved boards, holes of 5.0 mm (only 2018) were preferred. The bee species most frequently nesting in bamboo canes were *Ceratina cucurbitina* (Rossi) and *Ceratina dentiventris* Gerstacker, whereas in drilled logs *Hoplitis lepeletieri* (Pérez) was most common, but *Hoplitis adunca* (Panzer) was also recorded. Their abundance throughout the study period varied between species, and their role in biodiversity and sustaining wild flora is discussed.

Keywords: insect refuge; materials; wild bees; Ceratina cucurbitina; Ceratina dentiventris; Hoplitis lepeletieri

#### Introduction

Beneficial arthropods in agriculture include predators and parasitoids (Alford, 2019), but insect pollinators of crops and any other plants that require insects for reproduction can also be included in this term (Alford, 2019; Michener, 2007). Insect refuges can help to attract beneficial fauna to a particular place, and help to know the insect community that uses them (Maclvor, 2017). There are a number of peer-review publications on how to build insect refuges to harbour different types of bees and the use of them (Bosch, 1995; Fortel et al., 2016; Maclvor, 2017, who presents an extensive revision on the subject; Maclvor & Packer, 2015; Wilkaniec & Giejdasz, 2003), but useful insects such as butterflies, ladybugs, lacewings and others are not generally considered in studies related with insect refuges, although it is possible to find recommendations of materials and designs that could help to draw them to the refuges. There is a general consensus of a worldwide decline in pollinators, attributed to the chronic exposure of populations to a multitude of stressors such as habitat loss and resource availability, emerging viruses and parasites, exposure to pesticides, and climate change operating at various spatial and temporal scales (Becher et al., 2018; Burkle et al., 2013; Goulson, 2015; Potts et al., 2010). As insect pollinators are fundamental for the production and market value of many fruits, vegetable and field crops (Gallai et al., 2009; Klein et al.,

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2007), the importance of different methods that could mitigate their decline and support their populations are of increasing importance.

Ground nesting bees represent the majority of bee species and dominate in many open habitats, with all species of Andrenidae and Melittidae ground-nesting, as are most species of Halictidae and Colletidae (Michener, 2007). There is another group of bees, mainly Megachilidae and Apidae families, which make their nest either in pre-existing holes or dig their own cavities in firm substrate (e.g. pithy plant stems or soft wood) or the soil (Michener, 2007). Megachilidae and Apidae are the most important group of hymenopteran pollinators (specifically when crop pollination is involved) (Bosch & Kemp, 2002; Hansted et al., 2015; Miñarro & Twizell, 2015; Sedivy & Dorn, 2014).

Insect refuges are usually known as bee refuges, bee hotels, or cavity-nest boxes, and have different purposes and designs (Maclvor, 2017), with different pros and cons (Prendergast et al., 2020). They are most of the time (although not always) purposely designed to harbour wild solitary bees, which are very common and can play an important role in pollination (Brittain et al., 2013; Garibaldi et al., 2013; Holzschuh et al., 2012). The presence of different wild bee species depends on the geographical location and the crops and wild flowering plants present (Garratt et al., 2014). Adequate management of these wild flowering plants has been considered



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important to increase wild bee abundance and hence to have a positive effect on crop pollination (Gresty et al., 2018; Norfolk et al., 2016) and, in a wider point of view, if not direct pollinators of a particular crop, as part of a broader regional sustainability and conservation agenda (Wilson et al., 2018).

Most of the studies into the use of insect refuges (considered as bee hotels) have been carried out in northern regions of North America and Europe (Bosch & Kemp, 2002; Dainese et al., 2018; Fortel et al., 2016; Maclvor & Packer, 2015) and few studies have been conducted in southern regions, as for example in the Mediterranean basin. Using a particular zone in the southwest of Spain as a study area, we proposed two objectives in this study: 1) to construct insect hotels near to crops to test their use by the general beneficial arthropod fauna locally, but with a specific focus on bees, and 2) evaluate different materials cited in the literature and the species using them, improving the knowledge about both of them.

# Materials and methods

The study was carried out in the facilities of the ETSIA (University of Sevilla), located on the campus of Pablo de Olavide University (Dos Hermanas, Andalucia, Spain), with coordinates 37°21.173'N 5°56.323'W, and 20 m above sea level. Three sites were selected to place the refuges: a) a plot of arable land with annual crops, b) a plot with a collection of different fruit tree species, and c) a small garden  $(280 \text{ m}^2)$  with different perennial and deciduous plants. This third refuge was shadowed by an adjacent building until noon. In all cases, the refuges were facing south and were on top of concrete bricks around 20 cm above the ground. Distances between them varied from 140-200 m, with buildings and roads in-between. The surroundings can be considered peri-urban, with many arable croplands nearby, but also with residential areas and the university campus.

The climatic classification of Seville (SW of Spain, in Guadalquivir river valley) is 'Mediterranean Continental'. Considering the period from 1981 to 2010, the annual average temperature of the city is 19.2 °C, July being the hottest month (mean temperature of 28.2 °C, with max. temperature 36.0 °C and min. temperature 20.3 °C), and January the coldest month (mean temperature of 10.9 °C, with max. temperature 16.0 °C and min. temperature 5.7 °C) and 539 mm of annual rain (http://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos).

The refuges were constructed from 15 mm thick boards of phenolic okume plywood, resistant to outdoor conditions. The dimensions of the refuges varied: Refuge I – 600 mm (width) x 780/810 mm (height) x 200 mm (depth); Refuge 2 – 700 mm (width) x 720 mm (height) x 200 mm (depth); Refuge 3 – 800 mm (width) x 700 mm (height) x 200 mm (depth). The three refuges were designed with the same compartments (see Supplementary material 1), aimed to keep different materials that could harbour a variety of insects. Information about suitable materials for bee nesting (as cane stems, drilled wooden blocks, grooved boards), was obtained from different peer-reviewed publications (Bosch, 1995; Fortel et al., 2016; Maclvor, 2017; Wilkaniec & Giejdasz, 2003). The most unusual materials used in the refuges were included after consulting different web pages for gardening (e.g. https://garden-therapy.ca/build-a-bug-hotel/), with the objective of being used for sheltering/overwintering by other beneficial arthropods different from bees:

- Dedicated quarters to different groups of insects. There were three in each refuge, intended to provide shelter for a variety of insects different from wild bees. They were filled with straw and small branches of olive and fruit trees and had a lid to close the compartment with different openings to enter. One of the lids was painted in red as a lure for lacewings.
- Pine cones: A compartment of the refuges was filled with dried cones from pines (*Pinus pinea* L. and *Pinus halepensis* Mill.).
- Leaves and branches: A compartment of the refuges was filled with dead leaves from loquat (*Eriobotrya japonica* (Thunb.) Lindl) and small branches of olive and fruit trees.
- Arundo donax L. canes. A compartment was filled with Arundo canes, which varied in diameter (range 9.2 – 18.8 mm), but with a length of 19 cm.
- Bambusa sp canes. A compartment was filled with bamboo canes, which varied in diameter (range 1.3 8.1 mm), but with a length of 19 cm.
- Bricks: One or two bricks were put in each refuge, with holes of 25 mm diameter and filled with river sand.
- Logs: a compartment was filled with logs from the pruning of several trees (olive, apricot, plum), of 19 cm length and various thickness (3–10 cm), and with drilled holes (4.5–10 mm diameter and around 6 cm depth).
- Boards: Commercial boards of fir with no preserving treatments, with grooves of 3, 5, and 7 mm width, piled up to 8 layers thick in order to have a similar quantity of grooves of each dimension. Boards were of 190 mm length, 16 mm thickness, and variable width to adapt to the space available in each refuge.

The three refuges were put in their final location in November 2016 and filled with the various materials to study. There were two observation seasons: the first season was from March to October 2017, and the second season was from February to August 2018. At the end of each period, materials were recuperated and analysed in the laboratory. In November 2017, the refuges were refilled with new materials to be ready for the next season.

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|                                 | Bamboo<br>canes | Arundo<br>canes | Drilled<br>logs | Grooved<br>boards <sup>1</sup> | Leaves<br>and<br>branches | Bricks | Pine<br>cones | Quarters | Exterior | TOTAL |
|---------------------------------|-----------------|-----------------|-----------------|--------------------------------|---------------------------|--------|---------------|----------|----------|-------|
| Hymenoptera                     | 415             | 31              | 185             | 33                             | 267                       | I      | 2             | 337      | 49       | 1320  |
| Greater bees                    | 14              | 0               | 168             | 33                             | 0                         | 0      | 0             | 0        | 3        | 218   |
| Smaller bees                    | 338             | 0               | 10              | 0                              | 0                         | 0      | 0             | 0        | 3        | 351   |
| Carpenter bees                  | 0               | 31              | 0               | 0                              | 0                         | 0      | 0             | 2        | 3        | 36    |
| Social wasps                    | 52              | 0               | 0               | 0                              | 266                       | 0      | 2             | 333      | 29       | 682   |
| Other                           | 11              | 0               | 7               | 0                              | I                         | 1      | 0             | 2        | 11       | 33    |
| TOTAL OBSERVATIONS <sup>2</sup> | 418             | 31              | 194             | 36                             | 270                       | 5      | 2             | 344      | 189      | l 489 |

Table 1. Number of observations of different arthropods visiting the refuges during 2017 and 2018.

<sup>1</sup>Data only from 2018.

<sup>2</sup>Total Observations is the sum of Hymenoptera plus other arthropods and other non arthropod animals.

A record of the insects or other animals that could use the refuges and the materials in them was made (by JAHM) once a week, around noon, standing in front of each refuge for fifteen minutes at a distance of three metres. A camera was used to take photos of the refuge visitors, which helped with subsequent identification of the species. For observation purposes, hymenopterans visiting the refuges were classified visually as "greater bees", "smaller bees", "bumble/carpenter bees", and "social wasps". In the subsequent analysis of the individuals emerging from different materials, the range of length was quantified as 8-11 mm for "greater bees", and 5-7 mm for "smaller bees". Most of the Megachilidae finally identified (Anthidium sp., Hoplitis sp., Osmia sp.) were categorised as "greater bees", whereas Apidae (Ceratina sp.), some Megachilidae (Heriades sp., Megachile sp.), and all Vespidae (Eumeninae) were categorised as "smaller bees".

Materials from the refuges were thoroughly analysed in the laboratory (by JAHM, with the help of JEGZ) to examine which fauna could use them. All Bamboo canes, Arundo canes and grooved boards were opened to check their real use by the presence of brood cells, any other sign of use (debris of any type: exuvia, excrements, rests of pupae, etc), or the presence of individuals (for sheltering or overwintering), by hymenopterans or any other type of arthropods, whereas logs were only checked if they have the holes enclosed. Occupancy was calculated by counting all canes, grooves or holes used in any way (for nesting, with debris, for sheltering/overwintering) on the total available. All the materials (bamboo canes, grooved boards, and logs) with signs of nesting by bees were kept in an insectary at room temperature (no heating or cooling system used) in ventilated cages until adult emergence. Individuals found sheltering or overwintering were photographed, sometimes collected, and most of the cases given back to the refuge with the material in which were found. Francisco P. Molina (Departamento de Ecología Integrativa, Estación Biológica de Doñana (EBD-CSIC)) identified the most frequent bee species nesting in the materials when adult individuals emerged, in most cases several months after they were removed from the refuges. Specific keys were used to identify specimens of Apidae (Terzo et al., 2007; Terzo & OrtizSanchez, 2004) and Megachilidae (Benoist, 1940, 1931, 1929). In some cases, photographs of different insects taken in the refuges, or when individuals emerged from the materials, were uploaded in a specialised webpage (Biodiversidad Virtual, (https://www.biodiversidadvirtual. org/insectarium/) that helped to identify them (see Supplementary material 2). Voucher specimens are kept in the Department collection.

For the statistical analysis, Chi-square tests were applied to compare the level of use of the refuges and the use of the different materials in the two years. There were no replications of the refuges, so that conclusions obtained in this research must be considered as tentative.

# Results

Three refuges were installed, and the results obtained in the two years concluded that Refuge 3 was poorly used (Refuge 1: 1046 visits, Refuge 2: 382 visits, Refuge 3: 61 visits;  $X^2 = 1016.9$ ; df = 2, P < 0.001). Results presented below include the three refuges.

Considering the two years of study (Table 1), individuals from the Hymenoptera order represented 88.7% of the visitors to the refuges, whereas the other 11.3% comprised arachnids, dipterans, molluscs, and other groups. The most observed hymenopteran group was the social wasps (family Vespidae), with 682 observations, and the most common species identified was Polistes dominula (Christ, 1791), which formed its colonies mainly in the quarters enclosed with lids, and in the open compartment filled with leaves and branches. The hymenopterans with more interest, which can be assigned to the superfamily Apoidea as the most important group of pollinators, were denominated generically "greater bees", "smaller bees", and carpenter bees, and altogether they accounted for 605 observations (Table I). "Greater bees" preferred the holes drilled in logs, followed by grooved boards and bamboo canes. "Smaller bees" almost exclusively visited the bamboo canes, and carpenter bees were only observed sheltering in Arundo canes, which were the widest of all.

After being analysed in the laboratory for two years, the most occupied materials were bamboo canes,

|                       |                                   | 2017                |                 |                                   |                 |                 |                   |
|-----------------------|-----------------------------------|---------------------|-----------------|-----------------------------------|-----------------|-----------------|-------------------|
|                       | Bamboo<br>canes                   | Arundo<br>canes     | Drilled<br>logs | Bamboo<br>canes                   | Arundo<br>canes | Drilled<br>logs | Grooved<br>boards |
| NI                    | 647                               | 95                  | 254             | 569                               | 129             | 133             | 248               |
| % used                | 14.1                              | 1.1                 | 31.5            | 17.4                              | 2.3             | 37.6            | 8.5               |
|                       | $X^2 = 58.1$ ; d.f.= 2, P < 0.001 |                     |                 | $X^2 = 75.3$ ; d.f.= 3, P < 0.001 |                 |                 |                   |
| N2                    | 91                                | I                   | 80              | 99                                | 3               | 50              | 21                |
| % used by Hymenoptera | 74.7                              | 100                 | 100             | 89.9                              | 33.3            | 100             | 100               |
|                       | $X^2 = 2$                         | 23.6 ; d.f.= 2. P < | < 0.001         | X <sup>2</sup> =                  | =23.4 : d.f.= 3 | P < 0.001       |                   |

Table 2. Utilization of materials by different arthropods in the two years period after being analysed in laboratory.

NI represents the number of usable elements from each material that were analysed in the laboratory. For drilled logs and boards it represents the number of holes available.

N2 represents the number of elements from N1 identified as used (nesting, debris, sheltering/overwintering) in the laboratory.

Table 3. Distribution of the use (for nesting, with debris, or sheltering/overwintering) depending on the diameter of the material considered.

|                               | 20       | 17                            |          | 2018                          |          |                               |          |                  |          |
|-------------------------------|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|----------|------------------|----------|
| Bamboo canes                  |          | Drilled logs                  |          | Bamboo canes                  |          | Drilled logs                  |          | Grooved boards   |          |
| Diameter <sup>1</sup><br>(mm) | % of use | Diameter <sup>1</sup><br>(mm) | % of use | Diameter <sup>I</sup><br>(mm) | % of use | Diameter <sup>I</sup><br>(mm) | % of use | Diameter<br>(mm) | % of use |
| 1.8-2.6                       | 2.1      | 4.9-5.5                       | 35.0     | 1.3-2.5                       | 9.1      | 3.6-4.4                       | 12.2     | 3.0              | 4.8      |
| 2.7-3.4                       | 46.8     | 5.6-6.5                       | 46.3     | 2.6-3.3                       | 33.3     | 4.5-6.9                       | 16.3     | 5.0              | 85.7     |
| 3.5-4.2                       | 29.8     | 6.5-11.1                      | 18.7     | 3.4-4.2                       | 27.3     | 7.0-8.0                       | 34.7     | 7.0              | 9.5      |
| 4.3-4.9                       | 10.6     |                               |          | 4.3-5.0                       | 15.1     | 8.1-9.2                       | 36.7     |                  |          |
| 5.0-5.7                       | 8.5      |                               |          | 5.1-5.7                       | 11.1     |                               |          |                  |          |
| 5.8-8.I                       | 2.1      |                               |          | 5.8-7.4                       | 4.0      |                               |          |                  |          |

<sup>1</sup>Interval of diameters (lowest and highest diameter) within different categories.

Arundo canes, drilled logs and grooved boards. Both years showed that drilled logs were the most used material (Table 2), with occupancy rates of 31.5 and 37.6% in 2017 and 2018, respectively. Bamboo canes were the second material most used, with occupancy rates of 14.1 and 17.4% in 2017 and 2018, respectively. Grooved boards were less used, although there are data only from 2018, and when Arundo canes were opened they showed no clear sign of use for nesting purposes.

Hymenoptera was the insect order that showed a marked preference to nest (and sometimes shelter) in the materials which are the focus of this study (Table 2). Bamboo canes usage was mainly due to hymenopterans, which account for the 74.7 and 89.9% in the years 2017 and 2018, respectively, and the other occupants of bamboo canes were mainly Heteroptera and Coleoptera (adults and larvae of Dermestidae, and also some larvae and adults of Cleridae, with the species Trichodes octopunctatus (Fabricius, 1787) and Trichodes leucopsideus (Olivier, 1795). Drilled logs were not open, and the presence of some type of enclosure was interpreted as made by hymenopterans in the process of nesting, resulting in a 100% of use by them. When the grooved boards were dismounted, the presence of brood cells, or typical bee debris, indicated predominant usage by hymenopterans for nesting, although Coleoptera (larvae of Dermestidae) were also found. Arundo canes had a minimal use.

The materials tested had different inner diameters and their rates of use differed (Table 3). For bamboo canes, the most used diameters were in the range of 2.6/2.7 to 4.9/5.0 mm in both 2017 and 2018. Regarding drilled logs, holes with diameters between 4.9–6.5 mm were the most used in 2017, but in 2018 the most used holes were between 7.0–9.2 mm. In the boards, the most used holes were 5.0 mm in diameter (data only from 2018). Although Arundo canes were not used for nesting, their diameter range was 9.2–17.2 mm in 2017, and 9.3–18.8 mm in 2019.

After emerging from the different materials, or by direct observation, the species identified are shown in Table 4. The most common group of hymenopterans nesting in bamboo canes were of the Ceratinini tribe (included in family Apidae, subfamily Xylocopinae), belonging to the *Ceratina* species, accounting for the majority of individuals grouped as "smaller bees". The species identified were *C. cucurbitina* (Rossi, 1792) and *C. dentiventris* Gerstacker, 1869. They were observed visiting Refuges I and 2 (Figure IA–D), but particularly Refuge I during both years of study, and predominantly during warmer months. Other bee species that emerged from bamboo canes were Megachilidae such as *Heriades crenulatus* Nylander, 1856 and *Megachile apicalis* 

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| Material       | Family                            | Species                                   | Group          | Relative Abundance <sup>1</sup> (% |  |
|----------------|-----------------------------------|---|----------------|------------------------------------|--|
|                |                                   | 2017                                      |                |                                    |  |
| Bamboo canes   | Apidae                            | Ceratina cucurbitina (Rossi)              | Smaller bees   | 70                                 |  |
| Bamboo canes   | Megachilidae                      | Hoplitis lepeletieri (Pérez)              | Greater bees   | 15                                 |  |
| Bamboo canes   | Vespidae <sup>2</sup> (Eumeninae) | Leptochilus regulus (Saussure)            | Smaller bees   | 5                                  |  |
| Drilled logs   | Megachilidae                      | Hoplitis adunca (Panzer)                  | Greater bees   | 10                                 |  |
| Ū.             | C                                 | 2018                                      |                |                                    |  |
| Arundo canes   | Apidae                            | Xylocopa violacea (Linnaeus) <sup>3</sup> | Carpenter bees | I                                  |  |
| Bamboo canes   | Apidae                            | Čeratina cucurbitina (Rossi)              | Smaller bees   | 50                                 |  |
| Bamboo canes   | Apidae                            | Ceratina dentiventris Gerstacker          | Smaller bees   |                                    |  |
| Bamboo canes   | Megachilidae                      | Heriades crenulatus Nylander              | Smaller bees   | 10                                 |  |
| Bamboo canes   | Megachilidae                      | Megachile apicalis (Spinola)              | Smaller bees   | 5                                  |  |
| Bamboo canes   | Megachilidae                      | Anthidium sp                              | Greater bees   | 5                                  |  |
| Bamboo canes   | Vespidae <sup>2</sup> (Eumeninae) | Microdinerus sp                           | Smaller bees   | 5                                  |  |
| Bamboo canes   | Vespidae <sup>2</sup> (Eumeninae) | Leptochilus regulus (Saussure)            | Smaller bees   | 5                                  |  |
| Grooved boards | Megachilidae⁴                     | Anthidium sp                              | Greater bees   | 3                                  |  |
| Drilled logs   | Megachilidae                      | Hoplitis lepeletieri (Pérez)              | Greater bees   | 10                                 |  |
| Drilled logs   | Megachilidae                      | Hoplitis adunca (Panzer)                  | Greater bees   | 5                                  |  |
| Drilled logs   | Megachilidae                      | Osmia niveata (Fabricius)                 | Greater bees   | I                                  |  |

Table 4. Species of hymenopterans recovered/observed from the materials used.

<sup>1</sup>Values are an approximation of the relative abundance of the different species in the materials analysed in the laboratory and after emergence. <sup>2</sup>Not pollinators, but predators.

<sup>3</sup>Not nesting, only observed sheltering.

<sup>4</sup>Other Megachilidae were observed visiting and nesting in the boards, but were not recovered when the boards were open.

Spinola, 1808, which were also included in the category of "smaller bees". Other bee species identified after emerging from the bamboo canes, but in much lesser quantities, were *Hoplitis lepeletieri* (Pérez, 1879) (in 2017) and *Anthidium* sp (in 2018), both included in the group of "greater bees". Other hymenopterans identified after emergence were individuals of the subfamily Eumeninae (family Vespidae) as *Microdinerus* sp and *Leptochilus regulus* (Saussure, 1855).

The predominant species that emerged from drilled logs belonged to the family Megachilidae. The most frequent species was H. lepeletieri (in 2018), but Hoplitis adunca (Panzer, 1798) (in 2017 and 2018) and Osmia niveata (Fabricius, 1804) (in 2018) were also recorded. All of them were included in the category of "greater bees". Grooved boards were visited by different bees, all included in the category of "greater bees", but the only group that could be positively identified was Anthidium sp (also a Megachilidae), although photos of different individuals identified as belonging to the Megachilidae family were taken. Arundo canes were visited by different adults of the carpenter bee Xylocopa violacea (Linnaeus, 1758), but no trace of nesting was detected inside the canes when they were opened in the laboratory.

Refuges were visited for a long period of time in both study years, specially Refuges I and 2 (Figure I). The earliest visitor was the carpenter bee X. violaceae in 2018, when it used Arundo canes for sheltering in Refuge 2 from February to April (Figure ID). "Greater bees" were more common in Refuge 2 (Figure IC and ID), but were also observed in Refuge I, and in both 2017 and 2018 they appeared around April and lasted until June. "Smaller bees" were more common in Refuge I (Figure IA and IB) but were also present in high abundance in Refuge 2 in 2018, and were more common in warmer months (July, August, September) and even until October (in 2017).

### Discussion

Three refuges were installed and recorded: Refuges I and 2 were actively visited during the two years of the study, but Refuge 3 was almost ignored by all type of potential users. The failure in Refuge 3 could be for two reasons: firstly, the refuge was installed very near a building that shadowed the refuge until noon; and secondly, it was installed in a small garden planted with a variety of shrubs and trees, which as a whole had an ample blossom period, but the soil was covered with pine bark and consequently there was a total lack of weeds. As discussed below, most of wild bees that visited and bred in Refuges I and 2 were pollinators of wild plants, mainly annuals, so Refuge 3 may have been of little appeal to them.

The first objective of the insect refuges was to establish which arthropod species could use them in the environment of SW Spain. After two years of study, the most abundant group found in the refuges was Hymenoptera, predominantly represented by social wasps (P. dominula), but as they have very little interest as pollinators, they are not the focus of this work. They created their colonies in the compartments intended for different insect groups, which were loosely filled with straw, branches, and leaves. To prevent the presence of social wasps in insect refuges is highly recommended to fill all the compartments tightly, leaving no gaps that could use to construct their nests. The most interesting hymenopterans that used the refuges were those known as wild solitary bees, which can have an important role as pollinators, and so are the main interest of this work. One of the objectives of the study was

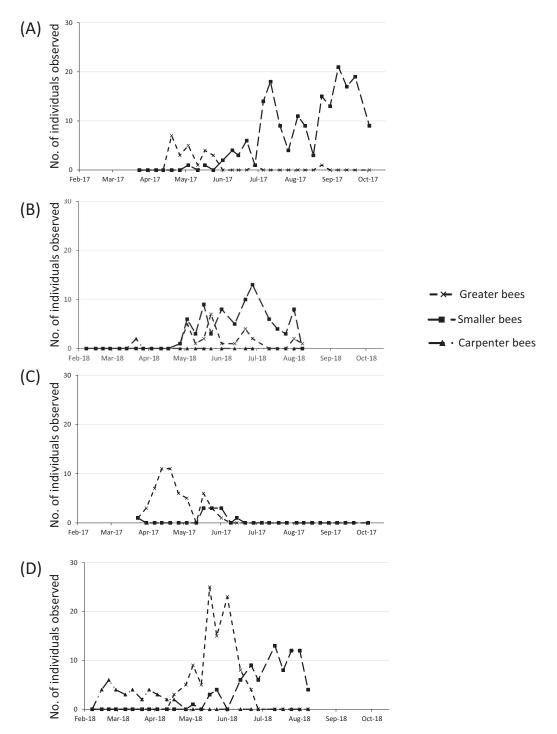


Figure 1. Seasonal patterns of bees using insect refuges in years 2017 and 2018. A) and B) Refuge 1; C) and D) Refuge 2.

to evaluate the use of the refuges by other beneficial insects (Neuroptera, Coccinellidae, Syrphidae, some Heteroptera) at least as sheltering, but no record of use by any of them was observed in the two years.

The second objective was to evaluate the best materials to use in the refuges. The refuges can be considered more properly as bee hotels and, as has been already noted (Bosch, 1995; Maclvor, 2017), materials such as different cane stems, drilled wooden blocks, grooved boards, are preferred for different groups of solitary bees. The other materials used in the study (pine cones, bricks, leaves and branches, straw in the specific quarters) were almost not used by any type of bee or beneficial insect, which stress the importance of selecting the materials accordingly with the objectives proposed and always with evidence-based recommendations.

The favoured materials used in this work were drilled logs and stems of *Bambusa* sp, and secondly grooved boards (although they had only one season of study). They had contrasting diameters, and together with preferences of the solitary bees, resulted in differential usage by different groups. The smaller diameters of bamboo canes were preferred by a group of species in which C. cucurbitina and C. dentiventris were the most common, but other species were identified, such as H. crenulatus and M. apicalis, all of which are pollinators of different plants (Molina & Bartomeus, 2019). The preferred diameters were from 2.6/2.7 to 4.9/5.0 mm. Drilled logs had a different range of hole diameters, and were preferred by other groups of species, mainly Megachilidae such as H. adunca, and other solitary bees, all known to be plant pollinators (Molina & Bartomeus, 2019). They preferably used diameters between 4.9 to 6.5 mm (2017), and 7.0 to 9.2 mm (2018). Finally, grooved boards were used by a variety of solitary bees, all of them Megachilidae, and the most used holes had a diameter of 5.0 mm.

The species observed visiting and nesting in the insect refuges are important in terms of knowing which hymenopterous fauna of SW Spain can use these bee hotels. The *Ceratina* species recorded in this work are considered to be common in southern Spain (Terzo & Ortiz-Sanchez, 2004). *Ceratina* species are known to be pollinators of different weeds and wild flowering plants in Spain (Lara-Ruiz, 2015), mainly Asteraceae and Labiatae. They are not regarded as important pollinators of horticultural crops (Ortiz-Sanchez & Belda, 1994), although in Pakistan there is a *Ceratina smarag-dula* (Fabricius, 1787) that is considered an efficient pollinator of alfalfa and other crops such as cucurbits (Ali et al., 2016).

The other hymenopterous species observed nesting in the refuges belonged mainly to the Megachilidae family, with an ample catalogue of species in Spain (Ornosa et al., 2006). The species observed in this work are not considered of importance for pollination of main crops such as citrus, almonds, peaches, plums, and other horticultural crops common in SW Spain (Ortiz-Sanchez & Belda, 1994), but they were observed visiting (although pollination was not confirmed) the wild flowering plants in the surroundings, as Asteraceae (Chrysanthemum coronarium L., Calendula arvensis (L.) Scop., Picris echioides L., Centaurea sp. and others) Convolvulaceae (Convolvulus arvensis L.), Cucurbitaceae (Ecballium elaterium (L.) A.Rich.) Brassicaceae (Sinapis alba L.), Papaveraceae (Papaver rhoeas L.), Malvaceae (Malva sylvestris L.), Fumariaceae (Fumaria sp.), Apiaceae (Daucus carota L.).

The species listed in Table 4 differ from the most commonly-mentioned species using cavity nests or bee hotels, such as Osmia bicornis (L), Osmia cornuta (Latreille), Osmia caerulescens (L), Megachile spp, or Hylaeus spp (Dainese et al., 2018; Fortel et al., 2016; Gresty et al., 2018), which can reflect the different distribution of species between regions of Europe. However, it must be taken into account the limited

area of study and the characteristics of the surroundings where the refuges were situated, which can influence the diversity of bee species present in the area. Besides, not all the bee species in the study area that can use cavity nests could be attracted to the refuges (Prendergast et al., 2020). Although the species listed in Table 4 are not regarded as important pollinators (http://apolo.entomologica.es/index.php?d=ranking), they can play an important role in the pollination of annual plants present in the area, which helps to support the natural populations of these plants and to keep biodiversity (Prendergast, 2020). This is important as these plants can be used as a natural cover crop in perennial crops typical in the area (e.g. olives, almonds, citrus); a cultural practice that is increasingly adopted due to its advantages (Alcántara et al., 2011; De Leijster et al., 2019; Porcel et al., 2013; Simoes et al., 2014). Xylocopa violacea, which was observed sheltering (but not nesting) in Arundo stems for a long period of time (from February to April), coincided with the blooming period of almonds, peaches, and other fruit crops near to the refuges, on which X. violacea adults were observed visiting the flowers. Although we had no record in our Arundo canes, this material has been used for nesting in other parts of the Mediterranean basin (Vicidomini, 1996).

The group denominated as "smaller bees" (mainly formed by *Ceratina* species) was most common in the period of warmer temperatures (in agreement with Terzo & Ortiz-Sanchez, 2004), when the predominant plant species in the surroundings of the refuges were Asteraceae and some Cucurbitaceae. "Greater bees" (with prevalence of Megachilidae) were more abundant in spring, when the flowering plant species were more diverse in the surroundings of the refuges.

Insect refuges were used for other hymenopterous species, including species of Eumeninae (family Vespidae), which are predators of different insect groups used to feed their progeny (Maclvor, 2017). Moreover, during the analysis of the material, the presence of some specific enemies of solitary bees was also recorded. The most abundant were larvae of the family Dermestidae (order Coleoptera), which usually behave as scavengers but can damage larvae and pupae of solitary bees (Bosch & Kemp, 2001; Krunic et al., 2005), and were very common in the grooved boards, but also present in bamboo canes. Secondly, some individuals from species of the family Cleridae (T. octopunctatus and T. leucopsideus, order Coleoptera) were also found, which larvae are noted predators of solitary bees progeny (Bosch & Kemp, 2001; Krunic et al., 2005), and finally, even individuals of the family Mutillidae (species Sigilla dorsata (Fabricius, 1798), from the order Hymenoptera), also predators of solitary bees progeny (Taylor et al., 2019), were observed entering the drilled logs.

Bee hotels may act as population sinks for bees by facilitating the increase of parasites, predators, etc (Maclvor & Packer, 2015) which are attracted to locations with high concentrations of their prey. For this reason, it is important to have careful management of bee hotels if they must play a significant role in sustainable agriculture (Mader et al., 2019)

In conclusion, the study presented here, although preliminary, shows that insect refuges were interesting to identify the variety of wild bee species that use cavity nests present in the area, it was useful to confirm the materials favourably used by these type of insects, and give different considerations to improve the use of refuges.

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#### Supplementary material

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