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Osmia cornuta Latr. (Hym., Megachilidae) as a potential pollinator in almond orchards

Releasing methods and nest-hole length

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Abstract

Three *Osmia cornuta* Latr. populations were released in each of three almond orchards near Reus (NE Spain). In each orchard, bees were offered the same choice of paper straws of four different lengths (12, 15, 18, and 21 cm) inserted in milk-carton nesting materials. Releasing methods used differed between orchards: bees of orchard A were released within their natal straws; bees of orchard B were released by inserting individual cocoons in new straws; bees of orchard C were mass-released in emergence boxes. Nesting bees at each orchard were counted, and the results showed that significantly more females dispersed in orchard C (79.10 %), than in orchard B (51.04 %) or orchard A (28.34 %). Females preferred to nest in longer straws (15 to 21 cm) than in 12 cm straws in the three orchards. In general, the number of cells built per nest increased, and sex ratio (males/females) of progeny decreased with straw length. Previously occupied straws were preferred to previously unoccupied straws in both orchards A and B. Comparison of these results with results obtained in other studies indicates that *O. cornuta* females select new holes when attractive nesting materials are offered, and previously occupied holes when less attractive nesting materials are available.

1 Introduction

Osmia cornuta Latr. is presently being studied as a pollinator of orchard crops in Yugoslavia (Krunic et al. 1990, 1991) and Spain (Bosch et al. 1992). *O. cornuta* is a close relative of *Osmia cornifrons* (Radoszkowski) and *Osmia lignaria propinqua* Cresson which have been established as managed pollinators in Japan and the US, respectively (Maeta and Kitamura 1974; Torchio 1993). The biologies of these three pollinators have proven to be very similar (Torchio 1987) but comparatively less effort has been made to experimentally test methods to improve management practices for *O. cornuta* (Torchio and Asensio 1985; Torchio et al. 1987).

In a previous study (Bosch 1994) the size of nesting shelters and their distribution throughout the orchard, and the preference of *O. cornuta* females for nest holes of different sizes was analyzed. Since results obtained in 1989 on female preference for holes of different lengths were not fully conclusive (Bosch 1994) this parameter was again tested in the present study. The preference for previously occupied holes versus previously unoccupied holes was also tested in the present study, and, furthermore, three different releasing methods were compared, to ascertain if they affected dispersal of pre-nesting females.

2 Materials and methods

In March 1991, three populations of 200 females and 400 males were released at each of three orchards planted to late-blooming almond cultivars near Reus (NE Spain). The first orchard (orchard A) was located in Perafort and contained 166 trees. The second orchard (orchard B) (196 trees) was an experimental orchard of the IRTA, and was located at Mas Valero. The third orchard (orchard C) (192

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trees) also belonged to the IRTA, and was located in the vicinity of Mas Bové Agricultural Research Station. The three orchards were more than 4 km away from one another.

Each orchard was supplied with a nesting shelter similar to those used in the 1989 and 1990 experiments (Bosch 1994), measuring 100 cm wide × 60 cm high × 40 cm deep. Inside each shelter four sets of milk carton nesting units (Torchio 1979, 1982; Bosch 1992 for further details) were placed. Each set was composed of 15 milk cartons with 15 paper straws each and two milk cartons with 25 paper straws each. All straws measured 8 mm inside diameter, but the straws of the first set measured 12 cm long, those of the second set 15 cm long, those of the third set 18 cm long, and those of the fourth set 21 cm long. These different types of nesting materials were placed inside the shelters following a Latin square distribution.

Bees used in this experiment were part of the progeny of bees released in Parc de Samà the previous year (Bosch 1994). They had been kept in their natal nests at room temperature for the summer, and in September 1991 they were x-rayed. The x-ray pictures were used to analyze the contents of the straws and three groups of approximately 124 straws containing 200 females and 400 males each were selected. These straws were over-wintered at 3 °C from October 30 1990 to March 2 1991.

On that day the first group of straws was inserted in orchard A nesting materials by introducing two natal straws in 11 milk cartons of each set, and one natal straw in three milk cartons of each set.

The second group of natal straws was dissected and cocoons were removed. Female cocoons were individually inserted in paper straws of each of the four above-mentioned lengths, and these seeded straws were inserted in orchard B nesting materials, so that 14 milk cartons of each set contained three seeded straws, and one milk carton of each set contained two. Male cocoons of this group were placed inside an emergence box (a cardboard box with holes on its sides through which bees could emerge).

The third group of natal straws was also dissected and both male and female cocoons were placed inside an emergence box, which was placed in the nesting shelter of orchard C (mass-release).

A trench 40 cm deep was dug in the ground beside each shelter to supply the bees with a source of moist soil to use as nesting material.

Since nesting *O. cornuta* females spend the night inside the nest they are provisioning, once the bees had already established, a flash light was used at night to count females nesting in each orchard.

At the end of the blooming season nesting materials were taken to the laboratory and kept at room temperature to allow for development of immatures. In September, straws were dissected and the composition of each nest was recorded.

3 Results and discussion

3.1 Emergence and nesting

On March 3, in sychrony with bloom initiation, all bees and nesting materials were taken to the respective orchards and placed in the nesting shelters. Some males and a few females emerged and deposited their meconium soon after they were released, but a spell of bad weather prevented bee activity until March 10, which was a sunny day. By then, blooming was well advanced. On that day many females could be seen entering and inspecting the holes in the nesting shelters, but no nest provisioning activities were observed. On March 14, females were already established, and the first plugged nests were spotted.

Females readily accepted the trenches dug near the shelters to collect moist soil, and foraged on almond flowers to collect pollen and nectar.

On April 2 there were no flowers left in the orchard and nesting materials were removed and taken to the laboratory. Ten females at orchard A, and three at orchard C were still provisioning nests. Therefore, the flying season lasted around a month.

3.2 Dispersal rates

Nocturnal counts of female bees inside their nests were made on March 15. In orchard A, 127 females were found "sleeping" in the paper straws. Since 13 adult females were found dead when straws were dissected in the autumn (6.5 % over-wintering mortality), the percentage of bees that dispersed or died before starting nesting activities (dispersal rate) was 32.1 %. Over-wintering mortality of population B was 4 %, and only 36 of the 192 females that emerged were found inside the straws, so dispersal rates were very high (81.25 %). Only four released females were found dead in the emergence box at orchard C

(2 % over-wintering mortality), but dispersal rates in this orchard were high, and as a result, only 41 females nested in the materials supplied (79.1 % dispersal). Differences in dispersal rates at the three orchards were highly significant (ch-sq = 127.56, df = 2, $p < 0.0001$).

When nesting materials were dissected in September, seven straws from orchard A, and 58 from orchard B contained no cocoons but had partially constructed cells at the bottom, indicating that nesting activities had started in these straws but had not progressed. Although some of these initiated nests could have been abandoned by bees that remained in the orchard, the majority of them were most likely started by females that later died or dispersed for unknown reasons. This must have been especially so in orchard B, where 58 such nests were recovered and only 36 females were found nesting. No such straws were recovered from orchard C. Additional evidence that more than 36 females started nesting activities at orchard B is found when the number of unplugged nests in each orchard is considered. Whereas only 3.93 % and 4.35 % of the nests with one or more cells recovered from orchards A and C respectively were unplugged (uncompleted), 63.29 % of those recovered from orchard B were. If dispersal rates are now re-calculated assuming that each nest with traces of bee activity was initiated by a different female, the percentage of females that dispersed without nesting was 28.34 % in orchard A, 51.04 % in orchard B, and 79.1 % in orchard C. Differences in dispersal rates calculated based on these figures are again statistically significant (ch-sq = 99.50, df = 2, $p < 0.0001$).

Although results on dispersal rates were somehow masked by sudden disappearence of nesting females at orchard B, a tendency for bees to return to their releasing point when they emerge within nesting materials was clearly expressed. These results coincide with those reported on *O. cornifrons* (MAETA 1978) and on *O. lignaria propinqua* (TORCHIO 1982) and can be most easily explained as a tendency of females to initiate nesting activities in the hole from which they emerged or nearby.

3.3 Utilization of straws of different lengths

The analysis of the nests recovered showed that 12 cm straws were less accepted as nesting material than longer straws in all three orchards (table 1) (A: ch-sq = 22.67, df = 3, $p < 0.001$; B: ch-sq = 18.28, df = 3, $p < 0.001$; and C: ch-sq = 8.35, df = 3, $p < 0.05$), but the three longest lengths were similarly accepted. Studies on other *Osmia* used for orchard pollination also indicate a preference for long over short holes (MAETA 1978; TORCHIO and TEPEDINO 1980). In general, the number of cells per nest increased with straw length (table 1), but this tendency was only statistically significant in orchard A (F = 11.23, df = 3, $p < 0.0001$), and not in orchards B (F = 1.556, df = 3, $p > 0.2$), and C (F = 2.32, df = 3, $p > 0.05$). In general, sex ratio of cells produced in each type of straw decreased with length (table 1), but the differences observed were only significant in orchard B (ch-sq = 13.49, df = 3, $p < 0.01$), and not in orchards A (ch-sq = 1.01, df = 3, $p > 0.7$), and C (ch-sq = 6.93, df = 3, $p > 0.05$). As a consequence of these results, the number of cells produced and the number of females produced tended to increase with straw length (table 1). These results paralel with results obtained in earlier studies with *O. cornuta* (BOSCH 1994). Therefore, 21 cm straws may be considered the most suitable straw length for *O. cornuta* propagation.

Rates of mortality due to developmental failure (causes unknown) did not differ between different types of straws in any orchard (A: ch-sq = 1.89, df = 3, $p > 0.7$; B: ch-sq = 4.74, df = 3, $p > 0.2$; and C: ch-sq = 5.41, df = 3, $p > 0.2$) (table 1), and rates of parasitism only differed between types of straws at orchard A (ch-sq = 8.33, df = 3, $p < 0.05$), and not at orchards B (ch-sq = 0.0005, df = 3, $p > 0.9$), and C (ch-sq = 0.67, df=3, $p > 0.9$). Most parasitism (73.68 %) was due to attack by the chalcid wasp *Monodontomerus obsoletus* Fab. and 26.32 % was due to the cleptoparasitic mite *Chaetodactylus osmiae* (Dufour). The flying season of *M. obsoletus* starts once the flying

Table 1. **Utilization of straws of different lengths by nesting *O. cornuta* females in three orchards in 1991**

Orchard	Length of straw (in cm)	Number available	Number utilized (%)	Cells produced	Cells/nest[1,2]	Females produced	Sex ratio (males/ females)	Developmental failure (%)	Parasitism (%)
A	12	275	36 (13.09)	140	3.89 ± 1.70	34	3.12	7 (5.00)	13 (9.29)
A	15	275	80 (29.09)	298	3.93 ± 2.04	74	2.99	26 (8.72)	33 (11.07)
A	18	275	72 (26.18)	291	4.04 ± 2.07	78	2.73	23 (7.90)	45 (15.46)
A	21	275	66 (24.00)	391	5.92 ± 3.03	108	2.62	31 (7.93)	34 (8.70)
B	12	275	6 (2.18)	18	3.50 ± 1.00	4	3.50	3 (16.67)	1 (5.56)
B	15	275	30 (10.91)	86	4.18 ± 2.14	28	2.07	5 (5.81)	7 (8.14)
B	18	275	23 (8.36)	56	3.25 ± 1.83	25	1.24	6 (10.71)	6 (10.71)
B	21	275	20 (7.27)	57	5.17 ± 0.75	34	0.68	2 (3.51)	6 (10.52)
C	12	275	13 (4.73)	40	3.25 ± 1.60	9	3.44	13 (32.50)	1 (2.50)
C	15	275	21 (7.64)	77	3.68 ± 2.19	12	5.42	23 (29.87)	1 (1.30)
C	18	275	29 (10.55)	119	4.18 ± 1.91	34	2.47	29 (24.37)	2 (1.68)
C	21	275	29 (10.55)	141	4.86 ± 2.28	44	2.25	26 (18.44)	3 (2.13)

[1] Only plugged nests. – [2] Mean ± Standard Deviation.

Table 2. **Utilization of previously occupied straws (natal straws [orchard A], seeded straws [orchard B]) and previously unoccupied straws (new straws [orchard A], unseeded straws [orchard B]) by nesting *O. cornuta* females in 1991**

Orchard	Type of straw	Number available	Number utilized (%)	Cells produced	Cells/nest[1,2]	Females produced/ available straw	Sex ratio (males/ females)	Developmental failure (%)	Parasitism (%)
A	Natal	124	88 (70.97)	319	3.63 ± 1.92	0.61	3.16	39 (12.26)	42 (13.17)
A	New	976	166 (17.01)	801	4.94 ± 2.58	0.22	2.67	48 (5.99)	83 (10.36)
B	Seeded	200	32 (16.00)	82	3.73 ± 1.35	0.21	1.00	7 (8.54)	7 (8.54)
B	Unseeded	900	47 (5.22)	135	4.22 ± 2.02	0.06	1.70	9 (6.67)	13 (9.63)

[1] Only plugged nests. – [2] Mean ± Standard Deviation.

season of *O. cornuta* is over, so parasitism by this wasp does not occur in the orchard but rather in the laboratory when *O. cornuta* larvae have already spun their cocoons. Therefore, it may be concluded that straw length had no effect on mortality.

3.4 Utilization of previously occupied and previously unoccupied straws

Straws from which bees had emerged (natal straws in orchard A, and seeded straws in orchard B) were preferred to previously unoccupied straws in both orchards A and B (ch-sq = 180.42, df = 1, $p < 0.0001$; and ch-sq = 28.51, df = 1, $p < 0.001$, respectively) (table 2). Conversely, in other experiments in which wood blocks were used as nesting material, both types of straws were equally accepted (BOSCH 1994) or new holes were preferred to natal holes (BOSCH 1992). When grooved boards made of K3 particle board were utilized as trap-nests (no female cocoons added), previously unoccupied holes were preferred to holes in which *O. cornuta* had nested the year before (BOSCH 1992). New holes seem to be preferred to old ones, but this may be compensated by a tendency of females to return to the hole from which they emerged. When nesting materials available are not very attractive (e.g. milk cartons [BOSCH 1992]) the latter tendency prevails, but when nesting materials are more attractive (e.g. wood blocks and grooved boards [BOSCH 1992]) new holes tend to be selected. Previously occupied holes were preferred to previously unoccupied holes in several orchard experiments with *O. lignaria propinqua* (TORCHIO 1976, 1981, 1982, 1984), but differences were greatest when the least attractive nesting materials were used, and lowest when the most attractive nesting materials were utilized (TORCHIO 1984).

Significantly less cells per nest were built in natal straws than in new straws in orchard A (t-Student = 4.12, df = 242, $p < 0.0001$), but not in orchard B (t-Student = 0.72, df = 27, $p > 0.4$) (table 2). Sonce female *O. cornuta* often do not empty nesting holes, but instead push remnants of old nests (soil, opened cocoons, feces) to the bottom of the hole, which results in a decrease in the space available, it is not surprising than fewer cells per nest were built in old straws in orchard A of this study and in earlier studies (BOSCH 1992).

The sex ratio of cells produced did not significantly differ between types of straws (ch-sq = 1.08, df = 1, $p > 0.2$ [orchard A], and ch-sq = 2.60, df = 1, $p > 0.1$ [orchard B]) (table 2). Parasitism was similar in both types of straws at both orchards (A: ch-sq = 1.80, df = 1, $p > 0.2$; B: ch-sq = 0.07, df = 1, $p > 0.7$), as was developmental failure in orchard B (ch-sq = 0.26, df = 1, $p > 0.9$), but developmental failure was significantly higher in natal straws than in new straws in orchard A (ch-sq = 12.37, df = 1, $p < 0.001$) (table 2). These results are similar to results obtained in previous studies in which wood blocks were used as nesting material (BOSCH 1992).

3.5 Cell production

Cell production in orchard A was 1120 cells, and of these, 294 were female cells. Therefore, 2.31 females were produced per nesting female. After deducting female mortality, we found that 227 females were obtained, and therefore, female population increased by 13.5 %. Male population in this orchard was increased by 70.25 %. Only 126 male cells and 91 female cells were constructed by bees nesting in orchard B (2.53 females produced per nesting female). Survival of females produced was 81.32 %, so only 74 females were obtained, which means a decrease in female population of 63 %. Male population in this orchard also decreased (73.25 %). In orchard C, where 278 male and 99 female cells were produced (2.41 females produced per nesting female), 67 females completed development, and as a result female population decreased by 66.5 % with respect to the initial population. Male population in orchard C decreased by 47 %.

This study differed from earlier studies (BOSCH 1994) in that blooming time available to bees for foraging in the orchards used in 1991 was shorter than in orchards used in 1989 and 1990, especially due to inclement weather at the beginning of the season. Under such circumstances it was expected that the reproductive potential of the *O. cornuta* populations released would be limited by pollen-nectar resources and decrease with respect to populations released in previous years. Furthermore, dispersal rates were purposedly encouraged in this study: first, milk cartons (the only nesting materials provided) have been proven to be much less attractive than other nesting materials such as wood blocks (Bosch 1992); and second, mass-release (Orchard C) clearly determined high rates of female dispersal in this orchard. However, even under such unfavorable conditions female production ranged from 2.31 to 2.53 female cells per nesting female, which should be enough to guarantee an increase in the bee population obtained with regard to the population initially released as long as dispersal and mortality rates are kept low through adequate managing practices. Such practices include long over-wintering periods, adequate distribution of nesting shelters, use of attractive nesting materials (Bosch 1994), release of populations within nesting holes, and control methods against *M. obsoletus* (Johansen and Eves 1969).

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Zusammenfassung

Osmia cornuta Latr. (Hym., Megachilidae) als möglicher Bestäuber in Mandelbaumkulturen. Freisetzungsmethoden und Nestlänge

In drei Mandelbaumkulturen in der Nähe von Reus (Nordostspanien) wurden *Osmia cornuta*-Populationen freigesetzt. In jeder Plantage wurden den Bienen Papierstrohhalme in 4 verschiedenen Längen (12, 15, 18 und 21 cm Länge) zum Nestbau angeboten. Die Freisetzungsmethoden unterschieden sich in den 3 Plantagen: in Plantage A wurden die Bienen in ihren Brutzellen ausgesetzt; in Plantage B wurden die Kokons in neue Strohhalme überführt und in diesen ausgesetzt; in Plantage C erfolgte die Freisetzung in Schlüpfboxen. In jeder Versuchsfläche wurden die nestbauenden Bienen gezählt. Die Ergebnisse zeigten, daß sich in C signifikant mehr Weibchen ausgebreitet hatten (79,10 %) als in B (51,04 % und in A (28,34 %). Die Weibchen bevorzugten zum Nestbau längere Strohhalme (15 bis 21 cm). Die Zahl der Zellen pro Nest stieg, und das Geschlechterverhältnis (Männchen/Weibchen) der Brut nahm mit zunehmender Länge der Strohhalme ab. In Fläche A und B wurden zuvor bereits genutzte Strohhalme neuen gegenüber bevorzugt angenommen. Der Vergleich dieser Ergebnisse mit denen anderer Versuche ergab, daß die Weibchen von *O. cornuta* neues Material für den Nestbau bevorzugen, wenn attraktives Material angeboten wird.

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