ORIGINAL ARTICLE

Osmia cornuta (Hymenoptera Megachilidae) densities required for apple pollination: a cage study

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SUMMARY

To estimate *Osmia cornuta* densities required for pollination of 'Braeburn' apple, trees were caged and supplied with different bee densities in 2000 and 2001. The following pollination treatments were compared: (1) one *O. cornuta* pair per five trees: 5 adjacent trees, 2 'Braeburn', 1 'Granny Smith' (pollenizer) and 2 'Braeburn', caged with 1 female and 1 male *O. cornuta*; (2) one *O. cornuta* pair per tree: 5 trees as above, caged with 5 female and 5 male *O. cornuta*; (3) open pollination: 5 trees as above, uncaged; (4) no pollinators: 5 trees as above, caged without pollinating insects. Fruitlet-set (fruitlets retained on the tree before June drop), misshapen fruit-percentages (percent fruits with at least one empty carpel), and seed-set (number of seeds per fruit) were measured. Cages with no pollinators had the lowest fruitlet-set (2000: 11.5%; 2001: 7.7%) and seed-set (2000: 1.6; 2001: 1.9), and the highest proportion of misshapen fruits (2000: 96.8%; 2001: 96.3%) in both years. Values obtained in cages with one *O. cornuta* pair per five trees were not significantly different from those obtained in open-pollinated trees. Cages with one *O. cornuta* pair per tree had fruitlet-set (2000: 37.9%; 2001: 20.4%) similar to cages with one pair per five trees (2000: 33.0%; 2001: 18.6%) or to open-pollinated trees (2000: 31.0%; 2001: 21.7%), but had significantly higher seed-set (2000: 7.5 vs. 5.7 and 6.0, respectively; 2001: 5.6 vs. 4.3 and 4.0, respectively) and lower misshapen fruit incidence (2000: 27.5% vs. 54.0% and 56.5%, respectively; 2001: 54.8% vs. 71.5% and 76.1%, respectively) in both years. One *O. cornuta* pair per five trees can provide commercially acceptable fruitlet-set, whereas one *O. cornuta* pair per tree can ensure maximum seed-set and thus high-quality yields on 'Braeburn.'

Keywords: *Osmia cornuta*, pollinator density, apple pollination, fruitlet-set, seed-set

INTRODUCTION

The fruit-set of most apple varieties depends on cross pollination, and thus on the synchronization of their bloom with that of a compatible variety and on pollinating insects (McGregor, 1976; Dennis, 1986; Free, 1993; Delaplane & Mayer, 2000). Although honey bee, *Apis mellifera* colonies are often used for pollination in apple orchards, several other species have been studied as alternative pollinators throughout the world. One of these species, the European *Osmia cornuta* flies early in the year (Tasei, 1973a), preferentially collects pollen from Rosaceae (Tasei, 1973a; Marquez *et al.*, 1994; Maccagnani *et al.*, 2003), and has a greater tolerance to inclement weather (Vicens & Bosch, 2000a; Ladurner *et al.*, 2000) and a higher rate of stigma contact than *A. mellifera* on orchard crops (Bosch & Blas, 1994a; Vicens & Bosch, 2000b). For these reasons, *O. cornuta* is, on an individual basis, a more effective pollinator than *A. mellifera* on fruit trees, as well as on other early blooming crops (Ladurner *et al.*, 2000, 2002). A series of studies on *O. cornuta* biology, ethology (Tasei, 1973a, 1973b; Bosch, 1994a), and rearing and releasing methods (Bosch, 1994b, 1994c; Bosch & Blas, 1994b; Krunic *et al.*, 1995; Ladurner *et al.*, 1999) have been conducted to develop a complete management system for this alternative pollinator species (review in Bosch & Kemp, 2002).

A critical aspect in pollination management is the density of pollinators required per plant or crop unit area (Free, 1993; Mantinger, 1998; Delaplane & Mayer, 2000). Excessive bee densities increase pollination costs. In addition, because *Osmia* species tend to have shorter foraging ranges than honey bees (Kitamura & Maeta, 1969; Vicens & Bosch, 2000c; Gathmann & Tscharntke, 2002; Maccagnani *et al.*, 2003) excessive densities may also result in pre-nesting female dispersal and higher progeny mortality due to insufficient pollen and nectar resources (Torchio, 1985; Bosch & Kemp, 2002).

Several methods can be used to estimate required bee densities. Direct methods are based on an assessment of fruit- and/or seed-set in caged plants visited by different numbers of bees or in fields supplied with different bee densities (Torchio, 1979; Torchio, 1985; Stern *et al.*, 2001). Indirect methods are based on estimates, such as required average number of bees per tree per minute (Mayer *et al.*, 1986; Stern *et al.*, 2001) or average number of flowers visited per nesting female in relation to the average number of flowers produced per tree (Bosch, 1994c; Maeta & Kitamura, 1981; Vicens & Bosch, 2000b). Computer models, based on honey bee density, among other variables, have been developed to predict fruit set in orchard crops (Brain & Landsberg, 1981; DeGrandi-Hoffman, 1983; DeGrandi-Hoffman *et al.*, 1986; Dennis, 1986).

As far as we know, previous studies on recommended *O. cornuta* densities for orchard pollination are all based on indirect methods (Bosch, 1994c; Vicens & Bosch, 2000b). Considering the average number of flowers visited per nesting female in relation to the average number of flowers produced per tree (approximately 2000 flowers per tree), in an estimate based on 2–3 visits per flower, Vicens & Bosch (2000b) concluded that

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one single *O. cornuta* female could adequately pollinate 3.3–5.5 'Red Delicious' trees. To test these estimates, we decided to use a direct method (cages supplied with different bee densities) to establish appropriate *O. cornuta* densities for the pollination of 'Braeburn' apple.

MATERIALS AND METHODS

After Vicens & Bosch (2000b), we investigated *O. cornuta* densities required for apple pollination on trees with approximately 2000 flowers per tree. Studies were conducted in 2000 and 2001 at the Agricultural and Forest Research Centre Laimburg, south of Bolzano northern Italy. The 3-year-old study orchard (11° East, 46° North, 220 m) measured 1.34 ha and was surrounded by a road to the north and east, and by other apple orchards to the south and west. 'Braeburn,' a self-sterile, early-blooming variety (Voltz *et al.*, 1996) was the main variety, and 'Granny Smith' the interplanted pollenizer. Each row had one 'Granny Smith' tree every 12–13 'Braeburn' trees. Distance between rows was 3.2 m and between trees within rows was 0.9 m. In the orchard there were neither *O. cornuta* shelters nor *A. mellifera* hives. Fruitlet-set (fruitlets retained on the tree before June drop per flowers produced), misshapen fruit-percentages (percentage of harvested fruits with at least one empty carpel), and seed-set (number of seeds per fruit), all considered the most reliable indicators of cross pollination (Sedgley & Griffin, 1989; Free, 1993; Mantinger, 1998), were measured on trees exposed to different pollination treatments with four replicates per treatment and per year:

1. One *O. cornuta* pair (one female + one male) per five trees. Five adjacent trees (two 'Braeburn,' one 'Granny Smith' and two 'Braeburn') were caged with one female and one male *O. cornuta*. Cage size was 3 × 4 × 3 m and screen mesh size 2 × 3 mm.
2. One *O. cornuta* pair per tree. Five trees were caged as above with five female and five male *O. cornuta*.
3. Open pollination. Five trees as above but without cage.
4. No pollinators. Five trees as above, caged without pollinating insects.

Each cage with *O. cornuta* individuals was provided with one nesting unit made of grooved wooden boards (Pinzauti, 1991; Krunic *et al.*, 1995) and one bundle of reed (*Arundo donax*, Poaceae) sections. Each grooved board unit had 56 nesting holes of 15 cm length and 8 mm diameter. Each reed section bundle had 20 reed sections of approximately 18 cm length and 8–10 mm diameter. At the first open flowers (on 9 April 2000 and 3 April 2001, respectively), *O. cornuta* cocoons that had been over-wintered at 4 °C for approximately 130 days were transferred to an incubator at 20 °C. At 10–15% bloom (11 April 2000 and 6 April 2001), the bees still inside their cocoons were brought to the orchard.

To verify whether all released *O. cornuta* females were present in the cages throughout the whole study period, every day we counted the number of females visiting flowers in each cage. To verify whether *O. cornuta* females nested in the cages, nesting materials were brought to the laboratory after bloom and the number of brood cells produced counted.

To determine fruitlet-set, in 2000, 50 flower buds were labelled on each 'Braeburn' tree when the blossoms were in the popcorn stage, and the number of flowers in each bud was recorded. Fruitlets on the labelled buds were counted on 5 May after unfertilized flowers had been shed (Sedgley & Griffin, 1989). In 2001, after flower drop (10 May), 100 flower buds (flower scars included) were selected randomly on each 'Braeburn' tree, and the number of fruitlets (0, 1, 2, etc.) on each flower bud counted and recorded with a handheld PC Husky fex21 (Itronix®). This recording method permits one to determine ratio of fruitlets per flower bud. To transform this value into fruitlet-set (fruitlets per flowers), we assumed that each flower bud produces five flowers (Sansavini, 1981; Bretaudeau & Fauré, 1991). At the end of May, crop load was reduced by hand thinning in 2000 and by chemical thinning (carbaryl) in 2001.

Higher seed-set results in better fruit development, shape and size, reduces rustiness, and increases sugar and calcium concentration in apple, all important for intrinsic fruit quality and storage (Marc, 1996a, 1996b; Volz *et al.*, 1996; Mantinger, 1998; Bruneau, 2000). To establish seed-set, we dissected harvested fruits and counted the number of well-formed seeds in each fruit. Aborted seeds with reabsorbed endosperm were not considered, whether or not the tegument was shrivelled. We also counted the number of fruits with at least one empty carpel. Because of the greater likelihood of fruits with empty carpels developing into misshapen fruits (McGregor, 1976; Free, 1993; Brault & de Oliveira, 1995; Delaplane & Mayer, 2000), we decided to address percentages of harvested fruits with at least one empty carpel as misshapen-fruit percentages.

Mean fruitlet-set, seed-set and misshapen fruit percentages were compared across treatments by means of one-way ANOVA. To improve homoscedasticity, fruitlet-set and misshapen fruit percentages were arcsine $\sqrt{x}$ transformed. The Newman Keuls Test ($P < 0.05$) was used for posthoc comparison of means. All analyses were performed with STATISTICA® 6.0. Means are reported with their standard error.

RESULTS

In both years, all bees emerged from their cocoons on the day of release. In each cage, all released *O. cornuta* females were observed visiting flowers throughout the whole study period (no mortality). The females provisioned several brood cells in each cage and year (table I).

There were significant differences in fruitlet-set, misshapen fruit-percentages, and seed-set across pollination treatments, both in 2000 (fruitlet-set: $F_{3,12} = 10.97$, $P < 0.001$; misshapen fruit-percentage: $F_{3,12} = 51.43$, $P < 0.0001$; seed-set: $F_{3,12} = 43.69$, $P < 0.0001$; fig. 1) and in 2001 (fruitlet-set: $F_{3,12} = 25.17$, $P < 0.0001$; misshapen fruit-percentage: $F_{3,12} = 57.91$, $P < 0.0001$; seed-set: $F_{3,12} = 32.43$, $P < 0.0001$; fig. 2). Cages with no pollinators had the lowest fruitlet-set and seed-set and highest proportion of misshapen fruits in both years. Values obtained in cages with one *O. cornuta* pair per five trees were not significantly different from those obtained in open-pollinated trees (figs 1 and 2). Cages with one *O. cornuta* pair per tree had similar fruitlet-set to cages with one pair per five trees or to open-pollinated trees, but had significantly higher seed-set and lower misshapen fruit incidence in both years.

DISCUSSION

Fruitlet- and seed-set in cages with one *O. cornuta* pair per five trees were similar to fruitlet- and seed-set in trees exposed to open pollination. We can assume open pollination was satisfactory because: (1) 20 *A. mellifera* hives had been placed 1 km from the study orchard and honey bees were abundant on 'Braeburn' flowers throughout the blooming period, and (2) fruitlet-set was high in open-pollinated trees (31.0% in 2000; 21.7% in 2001). Commercial fruitlet-set ranges from 10 to 30% in apple (Baldini, 1986; Dennis, 1986; Mantinger, 1998). One *O. cornuta* pair per five trees can therefore provide commercially acceptable fruitlet-set.

As already mentioned, considering the average number of flowers visited per nesting female in relation to the average number of flowers produced per tree, in an estimate based on 2–3 visits per flower, Vicens & Bosch (2000b) concluded that one single nesting *O. cornuta* female could adequately pollinate 3.3–5.5 'Red Delicious' trees. Even though the percentage of legitimate visits to apple flowers (percent flower visits with stigma contact) in *O. cornuta* is near 97.7% (Vicens & Bosch, 2000b), we agree with these authors that repeated stigma contact and flower visits are advisable for realistic estimates.

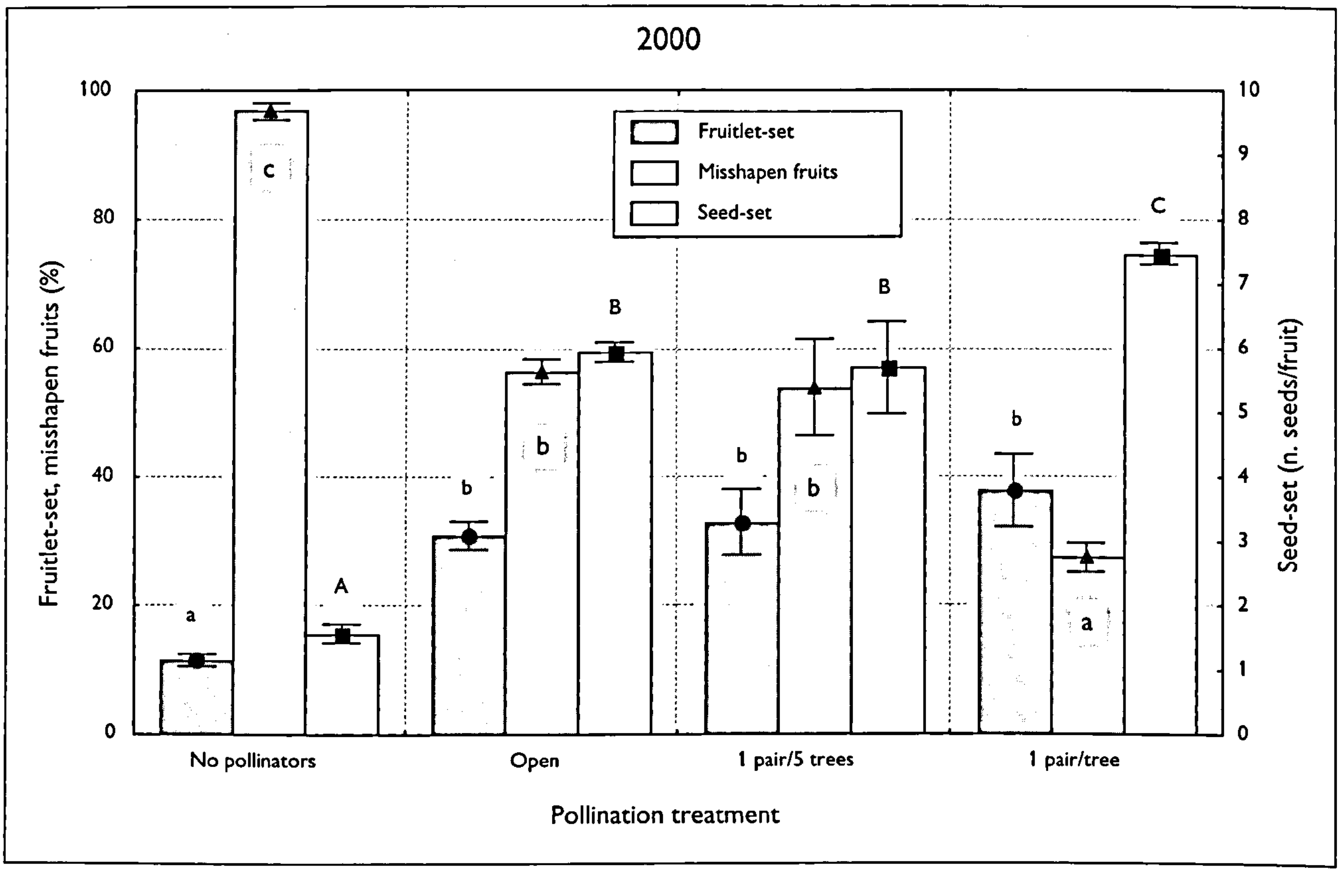

FIG. 1. Percentage fruitlet-set and misshapen fruits, and number of seeds per fruit (mean ± s.e.) on 'Braeburn' (n = 4) exposed to different pollination treatments (no pollinators, open pollination, one *Osmia cornuta* pair per five trees, and one *O. cornuta* pair per tree) in 2000. Different letters within the same type indicate significant statistical differences (Newman-Keuls Test $P < 0.05$).

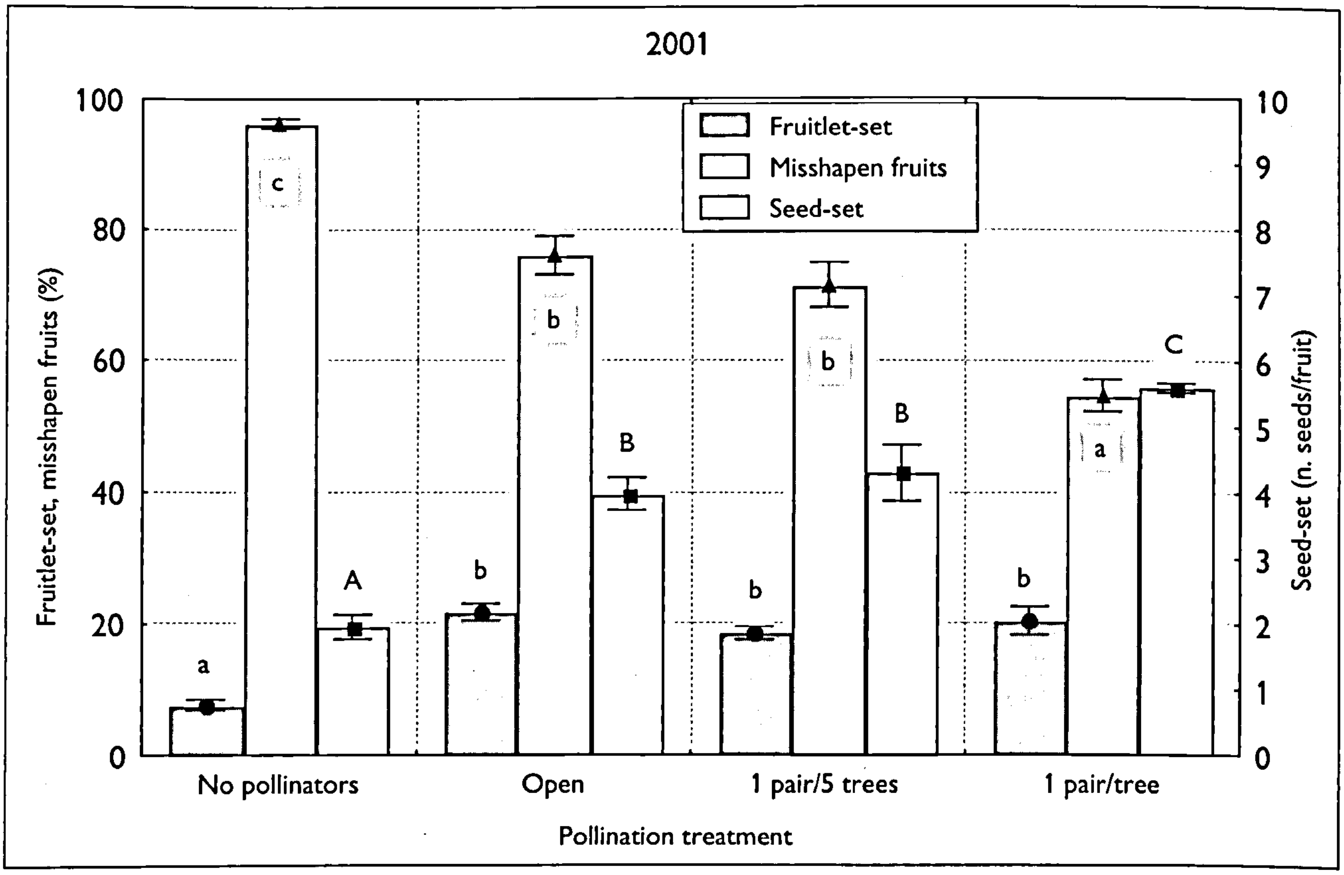

FIG. 2. Percentage fruitlet-set and misshapen fruits, and number of seeds per fruit (mean ± s.e.) on 'Braeburn' (n = 4) exposed to different pollination treatments (no pollinators, open pollination, one *Osmia cornuta* pair per five trees, and one *O. cornuta* pair per tree) in 2001. Different letters within the same type indicate significant statistical differences (Newman-Keuls Test $P < 0.05$).

TABLE 1. Mean number of brood cells produced (mean ± s.e.) in cages with trees pollinated by *Osmia cornuta* in 2000 and 2001.

Year	One *O. cornuta* pair per 5 trees	One *O. cornuta* pair per tree
2000	3.3 ± 1.0	5.8 ± 1.2
2001	5.0 ± 1.3	18.5 ± 3.6

According to Vincens & Bosch (2000b), the provisioning of *O. cornuta* female and male cells on apple requires on average 3911 and 2447 flower visits, respectively. In our cages with one *O. cornuta* pair per five trees we obtained a mean of 3.3 cells per cage in 2000 and 5.0 cells per cage in 2001. The infestation of several brood cells by pollen mites, *Chaetodactylus osmiae* (Chaetodactylidae), prevented us from establishing the progeny's sex ratio. If we assume only male cells were produced (lowest possible estimate), then females in the cages with one *O. cornuta* pair per tree would have made on average 8075 flower visits in 2000 and 12 235 in 2001. If we assume only female cells were produced (highest possible estimate), then females would have made 12 906 and 19 555 visits on average in 2000 and 2001, respectively. Each cage contained approximately 10 000 flowers (5 trees with approximately 2000 flowers per tree). Thus, the ratio visits per flower was 0.8–1.3 in 2000 and 1.2–1.9 in 2001; that is about half the ratio used by Vicens & Bosch (2000b) in their estimates. As with Vicens & Bosch (2000b), we did not include visits by males in these estimates.

By increasing bee density (one *O. cornuta* pair per tree), fruitlet-set did not increase, but a significant increase in fruit quality was obtained; in both years the percentage of apples with one or more empty carpels (percent misshapen fruits) decreased and seed-set increased. The mean number of brood cells produced per cage with one *O. cornuta* pair per tree was 5.8 in 2000 and 18.5 in 2001, respectively, which translates to an average of 14 193–22 684 and 45 269–72 353 flower visits per cage in 2000 and 2001, respectively, or 1.4–2.3 visits per flower in 2000 and 4.5–7.2 visits in 2001. These ratios are closer to (in 2000) or higher than (in 2001) the 2–3 visits used by Vicens & Bosch (2000b). Again, male visits were not included in our estimates.

Our results indicate that one *O. cornuta* pair per five trees provides commercially acceptable fruitlet-set, but one *O. cornuta* pair per tree can definitely ensure high seed-set and thus high-quality yields on apple. Thus, based on the number of flower visits per cage, more than one *O. cornuta* female per 3.5–5.5 trees, the density recommended by Vicens & Bosch (2000b), seem necessary for optimal pollination on apple. However, these authors based their estimates on an average number of 7.7 brood cells (2.8 female cells and 4.9 male cells) produced per female. Considering the average number of cells produced and the number of females released in each cage and year, the number of brood cells produced per female in our study was lower: it ranged from 1.2 to 5 cells per female. We think of two possible reasons for the lower number of cells produced per female:

1. Not all females were actively nesting; even though all released females were observed visiting flowers throughout the whole blooming period, some may not have established. These females, confined inside the cages, may have been visiting flowers only for nectar without actively provisioning brood cells and prone to disperse under open field conditions. Thus, our results are based on the number of flower-visiting females, whereas Vicens & Bosch (2000b) based their estimates on the number of actually nesting females.
2. Limited pollen-nectar resources; the number of flowers with available nectar and pollen varies during the day and over the blooming period (McGregor, 1976; Free, 1993; Maurizio & Schaper, 1994), and the likelihood of limited pollen-nectar resources is greater in cages than in the open field where females can forage on flowers other than those of the target crop, such as spontaneous flowers and flowers of nearby crops.

In 2001, fruitlet- and seed-set were lower and the proportion of misshapen fruits higher than in 2000, even though *O. cornuta* females were more active (more brood cells produced). The lower temperatures that occurred during bloom (2000: mean daily temperatures 8.2–18.3 °C, minimum temperatures > 5 °C; 2001: mean daily temperatures 5.8–15.2 °C, minimum temperatures < 5 °C nearly every day) may have affected the flowers and their reproductive organs without impairing the pollinating activity of *O. cornuta* females which are fully active from 10 to 12 °C (Bosch, 1994a; Vicens & Bosch, 2000a). Low temperature can result in low pollen production and ovule sterility, hinder pollen germination, pollen tube growth and fertilization, and reduce pollen viability (Baldini, 1986; Faust, 1989; Bubán, 1996; Mantinger, 1998).

In conclusion, we agree with Vicens & Bosch (2000b) that one single actively nesting *O. cornuta* female can adequately pollinate 3.3–5.5 apple trees with approximately 2000 flowers per tree. However, we think that in order to provide practical recommendations to growers on bee densities required for adequate apple pollination in the open field, population sizes need to be adjusted according to factors such as pre-nesting female dispersal, number of flowers per tree, number of trees per hectare, and the contribution to pollination of males and nectar-collecting females (Free, 1993; Bosch, 1994a; Vicens & Bosch, 2000b; Bosch & Kemp, 2002).

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