Original Article

# EFFECT OF POLLINATION BY THE *OSMIA BICORNIS* (SYN. *O. RUFA*) BEE ON FRUIT SET, SEED SET AND YIELD IN THREE APPLE CULTIVARS

Monika Fliszkiewicz* ORCID: 0000-0002-5913-3444
Karol Giejdasz ORCID: 0000-0001-8964-0466

Department of Apidology, Institute of Zoology, Poznan University of Life Sciences, Poland

*corresponding author: monika.fliszkiewicz@up.poznan.pl
Received: 10 July 2023; accepted: 24.10.2023

**Abstract**

Our study tested the pollination effectiveness of the megachilid bee *Osmia bicornis* L. (syn. *Osmia rufa*) on three apple cultivars (cv.): Lobo, Piros and Champion. The following pollination treatments were compared for each cultivar: insect pollination - caged trees with *Osmia bicornis* (syn. *Osmia rufa*) bees; open pollination - uncaged trees; self-pollination - caged trees without bees. Pollination success was measured based on the percentage of fruit set, the fruit yield per tree (measured as number of fruits and total fruit weight) and the number of seeds per fruit. Generally, there was a higher percentage of initial fruit-set and fruits at harvest on open-pollinated trees than on caged trees without bees, with an intermediate value recorded for caged trees pollinated by *O. bicornis* (syn. *O. rufa*). However, the fruit set on trees pollinated by *O. bicornis* (syn. *O. rufa*) was high and ranged from 49 to 69%, depending on the cultivar. The cv. Lobo trees pollinated by *O. bicornis* (syn. *O. rufa*) and open-pollinated trees produced higher fruit yields than without bees. Furthermore, the number of seeds per fruit was higher. Fruit yield and the number of seeds per fruit were higher for trees pollinated by *O. bicornis* (syn. *O. rufa*) than for caged trees without insects but lower than for open pollination trees of cv. Piros and cv. Lobo.

Keywods: apple, fruit yield, *Osmia bicornis*, pollination, seed number

## INTRODUCTION

Insect pollination determines the productivity of 75% of crop species grown on Earth (Kevan & Baker, 1983; Klein et al., 2007; Michener, 2007). Additionally, in recent years the production area for crops requiring pollination by insects has markedly increased, which in turn increases the importance of this ecosystem service for agriculture (Aizen et al., 2008). Horticulture and especially fruit growing are playing an increasingly important role in agricultural production. Nowadays, regions specializing in a specific type of fruit and vegetable production can be found in numerous countries.

Apple trees (*Malus* x *domestica* Borkh.) were known and used as early as 1000 BC (Juniper et al., 1999; Morgan & Richards, 2002; Cornille et al., 2014), and vast apple orchards were planted in ancient Europe and Asia (Cornille et al., 2014). Currently, most specialists believe that the genus *Malus* comprises 25-30 species and several subspecies (Robinson et al., 2001; Kellerhals, 2009). An apple, a fruit produced by the tree species *Malus domestica* from the rose family (*Rosaceae*), is the most important and most widely cultivated fruit in the world (Ferree & Warrington, 2003; Thamaraikannan et al., 2010; Czernyszewicz, 2016). According to the Food and Agriculture Organization of the United Nations (FAO), the largest producers of apples are China, USA, Turkey, Poland, Italy, France, Iran, Brazil, the Russian Federation, Chile and Argentina (FAO, 2018).

Fruit trees, including apple trees, are highly dependent on pollination by insects (Free, 1964; Delaplane & Mayer, 2000; Pardo & Borges, 2020), and only with sufficient pollination is it possible to obtain a high fruit yield of good commercial quality. In large commercial orchards, honeybees (*Apis mellifera*) are mainly used to improve crop productivity (Delaplane, 2000; Stern et al.,

2001), but wild insects are also known to play an important role in the pollination of apple trees. Some studies indicate that pollinators in apple orchards are represented mainly by bees (Hymenoptera: Anthophila) (Delaplane & Mayer, 2000; Stern et al. 2001; Garrat et al., 2014b). Notably, mason bees (e.g., *Osmia* spp.), mining bees (e.g., *Andrena* spp.) and bumblebees (*Bombus* spp.) have shown to be more efficient at pollinating apple trees than honeybees (Vicens & Bosch, 2000; Thomson & Goodell, 2001; Matsumoto et al., 2009; Martins et al., 2015; Pardo & Borges, 2020). Orchard pollination is very effective when a non-Apis species of commercial pollinators, such as bumblebees or mason bees, is used in addition to the honeybee (Brittain et al., 2013; Sapir et al., 2017). The advantage of the genus *Osmia* is that if the bees are provided with forage plants, they can develop independently and pollinate trees in the subsequent seasons (Sheffield et al., 2008). These arguments justify attempts to introduce *O. bicornis* (syn. *O. rufa*) in apple orchards to support the populations of native bees. The shorter flight range of wild bees, compared to that of honeybees, reduces the risk of fire blight transmission by pollinators and also facilitates the use of bees in pollination (Maccagnani et al., 2003).

In many countries around the world, controlled rearing of selected bees species from the genus *Osmia* is conducted according to their acceptance of various man-made nesting materials (Kemp, 2000; Monzón et al., 2004; Sedivy & Dorn, 2014).

The red mason bee is a managed pollinator widely used in fruit orchards in Europe. This bee species is well adapted to pollinating plants of the Rosaceae family and has a strong preference for Rosaceae pollen (Krunic & Stanisavljevic, 2006; Sedivy & Dorn, 2014). Its flight period lasts about two months, beginning in early April in Poland. During this period, female mason bees collect pollen and nectar, which are stored in the nest to create a series of nest cells partitioned with narrow mud walls. One female can produce a maximum of thirty cells in the nesting period (Tasei & Picart, 1973; Wójtowski, 1979). *O. bicornis* is a polylectic species, but one pollen type can dominate in the cells (Radmacher & Strohm, 2010).

The aim of the study was to verify the possibility of using *Osmia bicornis* (syn. *Osmia rufa*) to pollinate apple flowers through the assessment of how fruit setting, quantity and quality of the yield, and seed setting affects the pollination of three cultivars of apple trees: Lobo, Piros and Champion.

## MATERIAL AND METODS

The experiment was conducted from April to September 2019 in an apple orchard in Poland. The orchard covered an area of 3.75 h and had approximately 5,100 apple trees representing twenty-two cultivars. Fifteen trees comprising the cultivars Lobo, Piros and Champion, were selected for the experiment. Three variants of pollination were established for each of the cultivars. The first group included trees with restricted pollinator access during the bloom period - self-pollination, the second group of trees that could only be accessed by mason bees - pollination by mason bees, and the third group of trees could be freely accessed by various insect pollinators - open pollination. In order to eliminate the influence of other pollinators and evaluate the effectiveness of pollination by *Osmia bicornis* (syn. *Osmia rufa*), a cage test was used on the condition that the trees from the self-pollination and open pollination groups would constitute control groups. The trees in group 3 were not covered with cages, which allowed free access for numerous pollinator species. Before starting the experiment, all flower clusters and buds on each tree selected for the study were counted (Tab. 1). Trees from groups with *O. bicornis* (syn. *O. rufa*) and without bees were individually covered with cages at the beginning of their bloom period. In group without bees, cages prevented pollinators from accessing the trees. In group with bees, four mason bee pairs were placed in each cage. If any of the individuals died, it was replaced with a new one. Additionally, an artificial nest made of common reed stalks, 8-10 cm long and 0.6-0.8 cm in cross-section, was tied to the tree trunk with a wire.

Table 1.

Mean number of clusters per tree and mean number of flowers per cluster in the tested groups

| Pollination treatment | Lobo | | Piros | | Champion | |
|---|---|---|---|---|---|---|
| | clusters | flowers | clusters | flowers | clusters | flowers |
| **Selfpollination** | 44.0 | 5.6 | 34.8 | 4.5 | 127.8 | 4.3 |
| ***O. bicornis*** | 50.6 | 5.6 | 35.4 | 4.5 | 109.6 | 4.3 |
| **Open pollination** | 46.0 | 5.6 | 51.2 | 4.5 | 120.6 | 4.3 |

The 300 x 315 cm cages were made of mesh with the opening diameter of 1 mm. The base of each cage was a metal hoop with a diameter of 100 cm. The mesh below the hoop was tightly tied to the tree trunk. From the top, the cage was attached to the wire which was part of the orchard trellis. Additionally, every two or three days during the bloom period blooming branches that are considered effective pollenisers of the cultivars used in the experiment were placed in the cages. For cv. Lobo they were Gloster, Idared, Champion; for cv. Piros they were Champion and Delikates and for cv. Champion they were Delikates and Lobo. The cages were removed from the trees after they shed blossom. The fruit sets were counted on each tree before fruit drop. When the fruits ripened, the entire crop was harvested. The fruits from each tree were counted and weighed to the nearest 0.1 kg. Then, 10% of the fruits from each tree were randomly selected, and the numbers of seeds were counted after halving.

The effect of apple tree pollination by mason bees was assessed by determining:

- the percentage of initial fruits relative to the number of flowers per tree;
- the percentage of ripe fruits relative to the number of flowers per tree;
- the mean fruit weight and number of fruits per tree;
- the number of seeds per fruit.

In order to control the presence of insects that were important for open pollination, individual trees were observed at the beginning of the experiment. Two trees of each cultivar were randomly selected for testing from the rows containing trees from the experimental groups. Each of the trees was observed for ten minutes, and worker honeybees, bumblebees, wild bees and other insects were counted separately. The observations were repeated six times between 1:00 pm and 3:00 pm on sunny days, when the air temperature was above 15°C.

## STATISTICAL ANALYSIS

The Kolmogorov-Smirnov goodness-of-fit test was used for testing normality in all variables. The one-way analysis of variance was used to demonstrate the effect of the factor studied, and Duncan's test was performed to verify whether there were statistical differences between the average values experimental groups.

The number of seeds in the treatment groups was compared through the use of the Kruskal-Wallis test followed by the Bonferroni-Dunn post hoc test. The Statistica v.13 software was used to perform the analysis at a significance level $\alpha = 0.05$.

## RESULTS

**Initial fruit set (fruitlet set) and fruit set at harvest**

The fruitlet set and the pollination treatment were significantly related for all the cultivars tested (Fig. 1).

The fruitlet rate of cv. Lobo was higher for open-pollinated and caged trees pollinated by *O. bicornis* (syn. *O. rufa*) than for self-pollinated trees ($F=20.10$; $df=2, 12$; $p<0.001$).

In cv. Piros, the fruitlet rate was higher for open-pollinated trees than for self-pollinated trees, with an intermediate value recorded for caged trees pollinated by *O. bicornis* ($F=16.61$; $df=2, 12$; $p<0.001$). The fruitlet rate of cv. Champion was higher for open-pollinated trees than caged trees

pollinated by *O. bicornis* (syn. *O. rufa*) and self-pollinated trees (F=11.90; df=2, 12; p=0.0014).
The fruit set at harvest for all varieties also differed among the pollination treatments (Fig. 2). The fruit rate of cv. Lobo was higher for open-pollinated trees and those pollinated by *O. bicornis* (syn. *O. rufa*), than for self-pollinated trees (F=18.05; df=2, 12; p<0.001). The fruit rate of cv. Piros was higher for open-pollinated trees than for self-pollinated trees, with an intermediate value recorded for caged trees pollinated by *O. bicornis* (syn. *O. rufa*), (F=11.77; df=2, 12; p=0.001).
A similar relationship was noted in cv. Champion, but a significant difference was observed only between the fruit rate for open-pollinated trees and self-pollinated trees (F=11.90; df=2, 12; p=0.0014). In the case of open pollination, 56% of all insects visiting the flowers were honeybees, 22% wild bees, 10% bumblebees and 12% other insects, mainly Diptera flies.

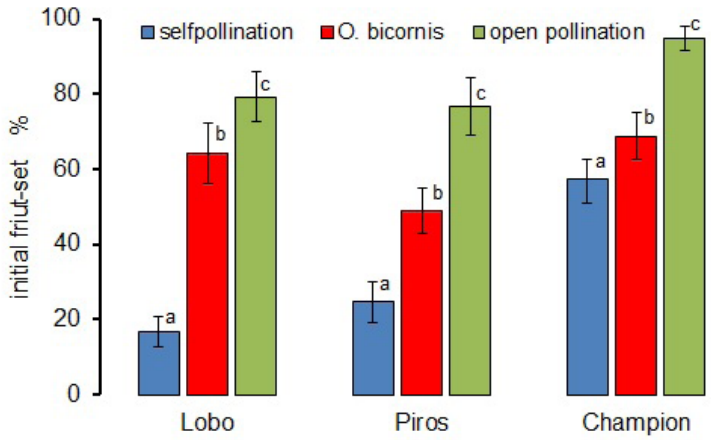



Fig. 1. Percent initial fruit-set of three apple cultivars exposed to different pollination treatments.

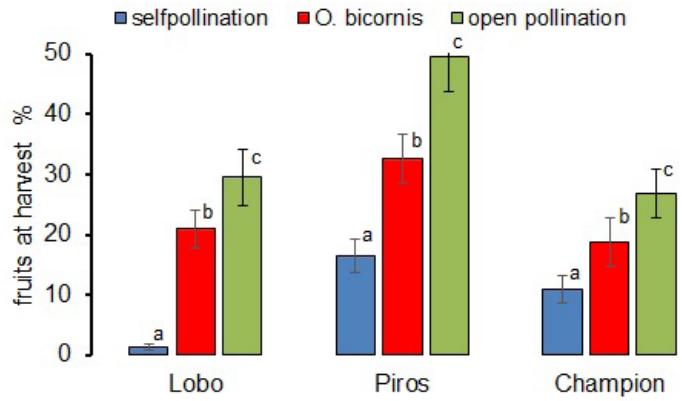



Fig. 2. Percent fruit-set at harvest of three apple cultivars exposed to different pollination treatments.

**Fruit number and fruit yield per tree**

The number of fruits obtained from one tree differed depending on the pollination treatment for all the cultivars tested (Fig. 3). In cv. Lobo, the mean fruit number per tree was higher for open-pollinated trees and those pollinated by *O.bicornis* (syn. *O. rufa*), than for self-pollinated trees (F=51.80; df=2, 12; p<0.001). The significant differences in the number of fruits depended on the pollination treatment variant in cv. Piros (F=26.81; df=2, 12; p<0.001) and cv. Champion (F=25.19; df=2, 12; p<0.001). The trees pollinated by *O. bicornis* (syn. *O. rufa*) had a significantly higher number of fruits than the caged trees with restricted bee access but lower than the open-pollinated trees.
The weight of fruit obtained from one tree and the pollination treatment were related for all the cultivars tested (Fig. 4). The mean weight of fruit per tree was higher for open-pollinated trees (8.7 kg) and those pollinated by *O.bicornis* (syn. *O. rufa*) (8.4 kg) than for self-pollinated trees (0.24 kg) in cv. Lobo (F=44.85; df=2, 12; p<0.001).
The fruit yield was higher for open-pollinated trees than for self-pollinated trees, with an intermediate value recorded for caged trees pollinated by *O. bicornis* (syn. *O. rufa*) in cv. Piros (F=10.02; df=2, 12; p=0.003) and cv. Champion (F=11.20; df=2, 12; p=0.002). The average yield of fruit harvested from one tree in cv. Piros ranged from 4.9 kg to 11.7 kg and in cv. Champion from 9.9 kg to 15.3 kg.

**Number of seeds per fruit**

The Kruskal-Wallis test showed a significant difference between the

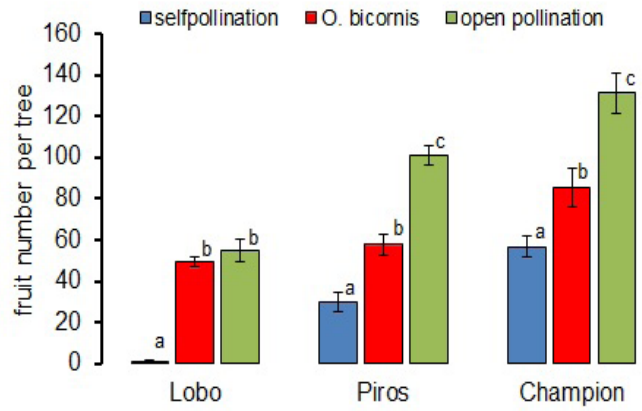



Fig. 3. The mean number of fruits harvested from one apple tree exposed to different pollination treatments.

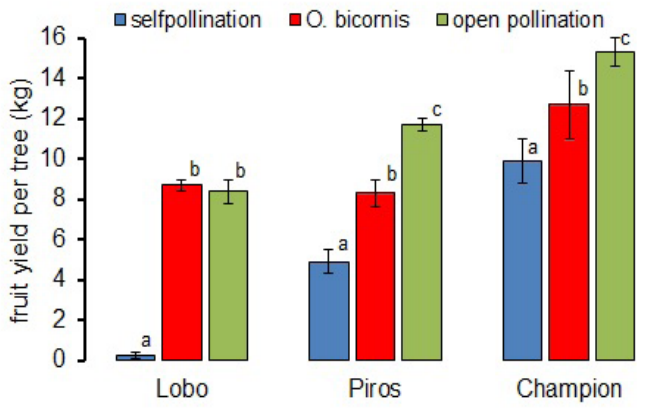



Fig. 4. The mean fruit yield (kg) harvested from one apple tree exposed to different pollination treatments.

treatment groups ($p < 0.001$) for all the cultivars tested (Fig. 5). The median seed number per fruit was higher for the open pollination treatment (10) and pollination by *O. bicornis* (syn. *O. rufa*) (10) than for self-pollination (1) in cv. Lobo ($H=33.624$; $df=2$, 68; $p<0.001$). The number of seeds for the self-pollination treatment ranged from 0 to 2 (3 in one fruit), and from 6 to 16 for the *O. bicornis* (syn. *O. rufa)* pollination and open pollination treatments (Fig. 5a).

There were significant differences between the treatment groups in cv. Piros ($H=46.172$; $df=2$, 85; $p<0.001$) and cv. Champion ($H=92.803$; $df=2$, 140; $p<0.001$). Pollination by *O. bicornis* (syn. *O. rufa*) caused the development of more seeds than self-pollination but less than the open pollination treatment in both cultivars (Fig. 5b, 5c). In cv. Piros, half of the fruits in the self-pollination group had no more than 0.5 seeds per fruit, in the *O. bicornis* (syn. *O. rufa*), group - 4, and in the open pollination group - 7. In cv. Champion, the median was: 2; 8; 13, respectively.

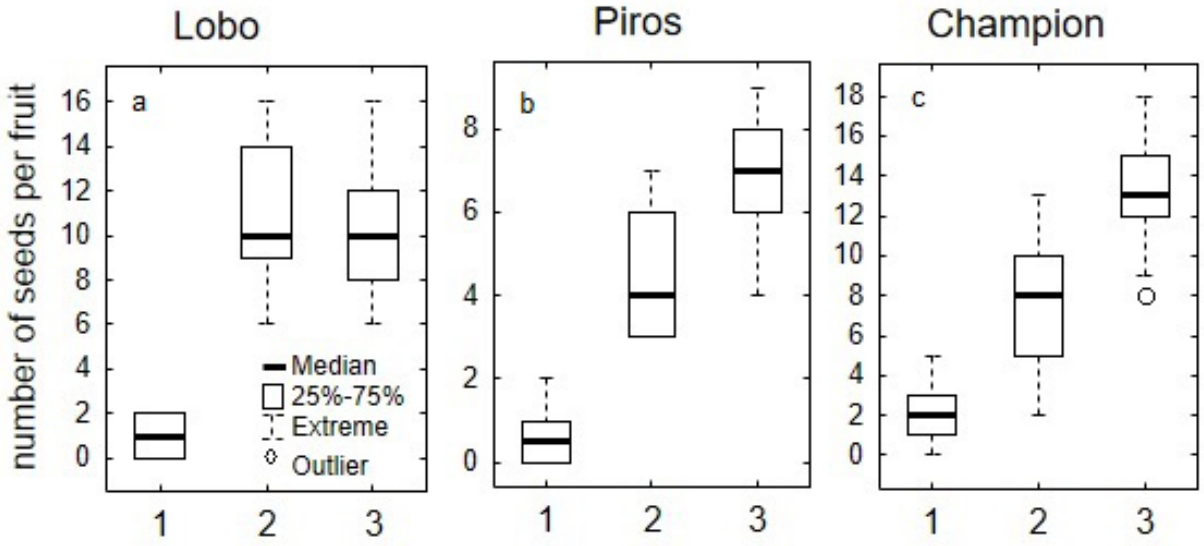



Fig. 5. The number of seeds per fruit of three apple cultivars exposed to different pollination treatments: 1) selfpollination, 2) pollination by *O. bicornis* (syn. *O. rufa*), 3) open-pollination.

## DISCUSSION

Most apple cultivars require cross-pollination with compatible pollen to ensure the profitability of commercial fruit cultivation, even with partially self-pollinating varieties. Therefore, cross-pollination with the participation of insect pollinators is believed to increase the fruit yield (Ramírez & Davenport, 2013). In general, more fruits are set and more seeds develop with open pollination than when trees are deprived of pollinators. Therefore, fruit set and seed set have been assumed to be the best indicator of pollination efficiency (Pardo & Borges, 2020).

The method of pollination influenced the number of fruit sets in all the apple cultivars examined. The highest share of fruitlets in relation to the number of flowers was observed in the open pollination group, then in the pollination by *O. bicornis* (syn. *O. rufa*) group, and the lowest was recorded in the self-pollination group of cv. Piros and Champion trees. On the other hand, fruit setting in cv. Lobo trees pollinated by *O. bicornis* (syn. *O. rufa*) and those exposed to open pollination was similar, whereas very few fruitlets developed in the self-pollination group.

Losada & Herrero (2013), Garratt et al. (2014b) and Jahed & Hirst (2018), indicate that with hand-pollination used to ensure the selection of an appropriate pollinizer, the proportion of fruit set to the total number of flowers ranges from 23% to 84%. Fruit setting in open pollination depends on native wild bees' presence, their abundance, species richness, functional group diversity or the use of managed pollinators i.e., honeybee accompanied by bumblebees and mason bees (Brittain et al., 2013; Mallinger & Gratton, 2015; Blitzer et al., 2016; Földesi et al., 2016; Sapir et al., 2017). The pollination success of apples has been found to be significantly related to the species richness of wild bees.

In open pollination of apple trees by managed mason bees *O. lignaria*, 25% of flowers develop fruit, and low temperature is the factor limiting their setting to 17% (Sheffield, 2014). On the other hand, Ladurner et al. (2004) demonstrated that fruit set determined on the basis of the number of fruitlets before June fruitlet drop, which is 22% and 31% depending on the season, is considered sufficient to obtain high commercial yields.

However, when there is a shortage of pollinators in orchards, open pollination of flowers yields up to 20% of fruit set when the measurement is performed during the first fruitlet thinning, or 15% when the measurement pertains to ripe fruit (Losada & Herrero, 2013; Garratt et al., 2014a; 2016). Regardless of the use of the honeybee as a support for little abundant wild pollinators, 11-18% of fruitlets remain on apple trees from three to four weeks after petal drop (Mallinger & Gratton, 2015).

In trees with no access to insect pollinators, fruit set is the lowest. Very slightly self-pollinating varieties produce no more than 2% of fruit (Fuji - 1%; Golden Delicious - 1.8%). A higher level of self-pollination was noted in cv. Elstar (7%), Cox and Gala (8-10%) and Idared (12.3%), while Cox's Orange Pippin (0.7-17%) is most variable in terms of this trait (Garratt et al., 2014b; De Witte et al., 1996).

In our experiment, caged trees from the self-pollination group presented 1.5-17% final fruit set after fruitlet drop in the three cultivars studied. Our study has proven that cv. Lobo has low potential for self-pollination and self-fertilization.

The efficiency of apple trees pollinated by *O. bicornis* (syn. *O. rufa*) measured as initial fruit-set (49-69%) and fruit-set at harvest (19-33%) can be considered satisfactory and sufficient to obtain a commercial fruit yield, although in two cultivars these values were lower than in the open pollination variant. Poorer pollination results may stem from the pollinisers' limited access to the flowers (Sharma et al., 2004).

The sources of compatible pollen in the cages were blooming branches of the polliniser's variety, which had a limited amount of pollen. The transfer of foreign pollen may have been difficult, because the branches of the polliniser were located at the same height as the tree trunk, and the insects flew more frequently in the upper part of the crown. The activity of the female bees in the cage was lower because they did not collect and carry pollen into the nest but only used it as their forage. However, the appropriate density of red mason bees in the cage leads to effective pollination of

flowers, resulting in fruitlet set (measured before June drop) of 20% and 38%, depending on the season (Ladurner et al., 2004).

A poorer pollination effect in the caged trees compared to open pollination may result from the presence of only one species of pollitaor. In open pollination, there is the synergy effect of various wild pollinator species or managed pollinators from the genus *Osmia* or *Bombus*, which are supported by the honeybee (Brittain et al., 2013; Földesi et al., 2016; Sapir et al., 2017). However, in one cage study, one *Osmia cornuta* male and one female per apple tree constituted the required density to obtain the maximum seed set and high-quality fruits of the 'Braeburn' variety (Ladurner et al., 2004).

Both fruit setting and seed development demonstrate the effectiveness of insect pollination. Seed setting and yield can reflect bees' contribution to pollination (Russo et al., 2017). This is a good measure of pollination efficiency because higher bee densities are required to obtain a high seed-set and lower the misshapen fruit incidence than to ensure proper fruit setting (Ladurner et al., 2004). Poor pollination in the early bloom period may reduce the number of seeds in the fruit (Volz et al., 1996). The seeds are formed as a result of the fertilization of ovules, which is preceded by pollination. Thus, the number of seeds in a fruit is dependent on the intensity of pollination, i.e. the number of pollen grains deposited on the stigma (Doust & Doust, 1988).

In most varieties of apples, the fruit has five carpels, each with two seeds. Sometimes the fruit has 6 carpels, or a carpel develops three seeds. The Northern Spy cultivar has four ovules that can develop into up to twenty seeds (Delaplane & Mayer, 2000).

Following hand-pollination of different apple cultivars with foreign pollen, 5.4-8.6 seeds (Jahed & Hirst, 2018) or 3.5-4 seeds develop in the fruit (Garratt et al., 2014b). When *O. lignaria* are used in the orchard for pollinating flowers, the fruits of cv. McIntosh and Cortland contain from six to eight seeds (Sheffield, 2014). Fewer seeds develop when pollinator access is restricted, less than one seed in one fruit on average. When there is a shortage of pollinators, 1.5-2.5 seeds per fruit are obtained, even in open pollination (Garratt et al., 2014b). In the present study, fruits from trees pollinated by *O. bicornis* (syn. *O. rufa*) had the maximum number of seeds in cv. Lobo. In the case of cv. Champion and Piros, the number of seeds was higher than in the self-pollination variant and comparable to the results of cross-pollination obtained by the authors mentioned above.

Seed setting is an important indicator of apple fruit development and is associated with marketable yield and high-quality fruit. Apples with a high seed count are larger and have a better shape and taste than the misshapen ones (Brault & Oliveira, 1995; Keulemans et al., 1996; Buccheri & Vaio, 2005). It is assumed that at least from six to eight ovules must be fertilized so that the fruit is not misshapen. This means that misshapen fruits have two or more empty carpels (Bralt & de Oliviera, 1995; Sheffield, 2014). Thus, fruit development is stimulated by pollination, pollen germination and seed-derived phytohormones (Dust & Dust, 1988). The apple fruit develops mainly from the receptacle, and proper fruit development has an impact on the marketable yield (Shivanna & Tandon, 2014).

Trees pollinated by *O. bicornis* (syn. *O. rufa*) produced a better yield than the caged trees with restricted bee access. In general, fruit yield, fruit set and seed number were all similarly dependent on the pollination method. The use of *O. bicornis* (syn. *O. rufa*) for pollination increases fruit set and yield, as well as the number of seeds in the fruit. Introducing *O. bicornis* (syn. *O. rufa*) to an apple orchard as a managed pollinator can improve pollination effects and fruit quality. The red mason bee is an effective pollinator of apple trees and could be used as a managed wild bee, which would be a complementary element of the pollinator community in orchards.

**ACKNOWLEDGMENTS**

Research conducted with the statutory funding No. 506-511-04-00 the Faculty of Veterinary Medicine and Animal Science Poznan University of Life Sciences, Poland.

**REFERENCES**

Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., Klein, A.M. (2008). Long-Term Global Trends in Crop Yield and Production Reveal No Current Pollination Shortage but Increasing Pollinator Dependency. *Current Biology, 18,* 1572-1575. https://doi.org/10.1016/j.cub.2008.08.066

Blitzer, E.J., Gibbs, J., Park, M.G., Danforth, B.N. (2016). Pollination services for apple are dependent on diverse wild bee communities. *Agriculture, Ecosystems & Environment, 221,* 1-7. https://doi.org/10.1016/j.agee.2016.01.004

Brault, A.M., & de Oliveira, D. (1995). Seed Number and an Asymmetry Index of McIntosh'Apples. *American Society for Horticultural Science, 30*(1), 44-46. https://doi.org/10.21273/HORTSCI.30.1.44

Brittain, C., Williams, N., Kremen, C., Klein, A. (2013). Synergistic effects of non-Apis bees and honey bees for pollination services. *Proceedings of the Royal Society B. Biological Sciences, 280*(1754), 20122767. https://doi.org/10.1098/rspb.2012.2767

Buccheri, M., & Di Vaio, C. (2005). Relationship among seed number, quality, and calcium content in apple fruits. *Journal of Plant Nutrition, 27*(10), 1735-1746. https://doi.org/10.1081/PLN-200026409

Cornille, A., Girau, T., Smulders, M.J.M., Roldán-Ruiz, I., Gladieux, P. (2014). The domestication and evolutionary ecology of apples. *Trends in Genetics, 30*(2), 57-65. https://doi.org/10.1016

Czernyszewicz, E. (2016). Long-term trends in production and consumption of apples in Poland, Europe and worldwide. *Acta Scientiarum Polonorum Hortorum Cultus, 15*(3), 95-104.

De Witte, K., Vercammen, J., van Daele, G., Keulemans, J. (1996). Fruit set, seed set and fruit weight in apple as influenced by emasculation, self-pollination and cross-pollination. *Acta Horticulturae, 423,* 177-183. https://doi.org/10.17660/ActaHortic.1996.423.23

Delaplane, K., & Mayer, D. (2000). Crop Pollination by Bees. Wallingford, UK.

Doust, J.L., & Doust, L.L. (1988). Plant reproductive ecology: patterns and strategies. Oxford: University Press on Demand.

FAO (Food and Agriculture Organization of the United Nations) (2019). Food and Agriculture Organization of the United Nations-FAOSTAT

Ferree, D.C., & Warrington, I.J. (2003). Apples: Botany. Production and Uses., New York: CABI

Földesi, R., Kovács-Hostyánszki, A., Kőrösi, Á., Somay, L., Elek, Z., Markó, V., Báldi, A. (2016). Relationships between wild bees, hoverflies and pollination success in apple orchards with different landscape contexts. *Agricultural and Forest Entomology, 18*(1), 68-75. https://doi.org/10.1111/afe.12135

Free, J.B. (1964). Comparison of the importance of insect and wind pollination of apple trees. *Nature, 201*, 726-727.

Garratt, M.P.D., Breeze, T.D., Boreux, V., Fountain, M.T., Mckerchar, M., Webber, S.M., Biesmeijer, J.C. (2016). Apple pollination: demand depends on variety and supply depends on pollinator identity. *PloS One, 11*(5), e0153889. https://www.ncbi.nlm.nih.gov/pubmed/27152628 doi: 10.1371/journal.pone.0153889

Garratt, M.P.D., Truslove, C.L., Coston, D.J., Evans, R.L., Moss, E.D., Dodson, C., Potts, S.G. (2014a). Pollination deficits in UK apple orchards. *Journal of Pollination Ecology, 12*(2), 9-14. https://doi.org/10.26786/1920-7603(2014)8

Garratt, M.P., Breeze, T.D., Jenner, N., Polce, C., Biesmeijer, J.C., Potts, S.G. (2014b). Avoiding a bad apple: Insect pollination enhances fruit quality and economic value. *Agriculture, Ecosystems and Environment, 184*, 34-40. https://doi.org/10.1016/j.agee.2013.10.032

Jahed, K.R., & Hirst, P.M. (2018). Pollen source effects on seed number, fruit quality and return

bloom of apple. *Journal of American Pomological Society, 72*(4), 212-221.

Juniper, B.E., Watkins, R., Harris, S.A. (1999). The origin of the apple. *Acta Horticulturae, 484,* 27-33. https://doi.org/10.17660/ActaHortic.1998.484.1

Kemp, W.P. (2000). The future of crop pollination. *American Bee Journal, 140*(11), 851-853.

Keulemans, J., Brusselle, A., Eyssen, R., Vercammen, J., Van Daele, G. (1996). Fruit weight in apple as influenced by seed number and pollinizer. *Acta Horticulturae, 423,* 201-210. https://doi.org/10.17660/ActaHortic.1996.423.26

Kevan, P.G., & Baker, H.G. (1983). Insects as flower visitors and pollinators. *Annual Review of Entomology, 28,* 407-53. https://doi.org/10.1146/annurev.en.28.010183.002203

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T. (2007) Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences, 274*(1608), 303-313. https://doi.org/10.1098/rspb.2006.3721

Krunic, M., & Stanisavljevic, L. (2006). Augmentation of managed populations of *Osmia cornuta* and *Osmia rufa* (Hymenoptera: Megachilidae) in Southeastern Europe. *European Journal of Entomology, 103*(3), 695-697.

Ladurner, E., Recla, L., Wolf, M., Zelger, R., Burgio, G. (2004). *Osmia cornuta* (Hymenoptera Megachilidae) densities required for apple pollination: a cage study. *Journal of Apicultural Research, 43*(3), 118-122. https://doi.org/10.1080/00218839.2004.11101121

Losada, J.M., & Herrero, M. (2013). The influence of the progamic phase for fruiting in the apple tree. *Annals of Applied Biology, 163*(1), 82-90. https://doi.org/10.1111/aab.12035

Maccagnani, B., Ladurner, E., Santi, F., Burgio, G. (2003). *Osmia cornuta* (Hymenoptera, Megachilidae) as a pollinator of pear (*Pyrus communis*): fruit-and seed-set. *Apidologie, 34*(3), 207-216. https://doi.org/10.1051/apido:2003009

Mallinger, R.E., & Gratton, C. (2015). Species richness of wild bees, but not the use of managed honeybees, increases fruit set of a pollinator-dependent crop. *Journal of Applied Ecology, 52*(2), 323-330. https://doi.org/10.1111/1365-2664.12377

Martins, K.T., Gonzalez, A., Lechowicz, M.J. (2015). Pollination services are mediated by bee functional diversity and landscape context. *Agriculture, Ecosystems and Environment, 200*, 12-20. https://doi.org/10.1016/j.agee.2014.10.018

Matsumoto, S., Abe, A., Maejima, T. (2009). Foraging behavior of *Osmia cornifrons* in an apple orchard. *Scientia Horticulturae, 12*(1), 173-179. https://doi.org/10.1016/j.scienta.2009.01.003

Michener, C.D. (2007). The Bees of the World. Second Edition. Johns Hopkins University Press, Baltimore, Maryland.

Monzón, V.H., Bosch, J., Retana, J. (2004). Foraging behavior and pollinating effectiveness of *Osmia cornuta* (Hymenoptera: Megachilidae) and *Apis mellifera* (Hymenoptera: Apidae) on "Comice" pear. *Apidologie, 35*(6), 575-585. https://doi.org/10.1051/apido:2004055

Morgan, J., & Richards, A. (2002). The new book of apples. Ebury Press, London

Pardo, A.; & Borges, P.A.V. (2020). Worldwide importance of insect pollination in apple orchards: A review. *Agriculture, Ecosystems & Environmen, 293*, 106839. https://doi.org/10.1016/j.agee.2020.106839

Radmacher, S., & Strohm, E. (2010). Facteurs affectant la taille corporelle de la progéniture de l'abeille soliter *Osmia bicornis* (Hymenoptera : Megachilidae). *Apidologie, 41*, 169-177. https://doi.org/10.1051/apido/2009064

Ramírez, F., & Davenport, T.L. (2013). Apple

pollination: a review. *Scientia Horticulturae, 162,* 188-203. https://doi.org/10.1016/j.scienta.2013.08.007

Robinson, J.P., Harris, S.A., Juniper, B.E. (2001). Taxonomy of the genus Malus Mill. (Rosaceae) with emphasis on the cultivated apple, *Malus domestica* Borkh. *Plant Systematics and Evolution, 226*, 35-38. https://doi.org/10.1007/s006060170072

Russo, L., Park, M.G., Blitzer, E.J., Danforth, B.N. (2017). Flower handling behavior and abundance determine the relative contribution of pollinators to seed set in apple orchards. *Agriculture, Ecosystems and Environment, 246*, 102-108. https://doi.org/10.1016/j.agee.2017.05.033

Sapir, G., Baras, Z., Azmon, G., Goldway, M., Shafir, S., Allouche, A., Stern, R.A. (2017). Synergistic effects between bumblebees and honey bees in apple orchards increase cross pollination, seed number and fruit size. *Scientia Horticulturae, 219*, 107-117. https://doi.org/10.1016/j.scienta.2017.03.010

Sedivy, C., & Dorn, S. (2014). Towards a sustainable management of bees of the subgenus *Osmia* (Megachilidae; Osmia) as fruit tree pollinators. *Apidologie, 45*(1), 88-105. https://doi.org/10.1007/s13592-013-0231-8

Sharma, H.K., Gupta, J.K., Thakur, J.R. (2004). Effect of bee pollination and polliniser proportion on apple productivity. In *VII International Symposium on Temperate Zone Fruits in the Tropics and Subtropics, 662,* (p. 451-454). https://doi.org/10.17660/ActaHortic.2004.662.68

Sheffield, C.S. (2014). Pollination, seed set and fruit quality in apple: studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Annapolis valley, Nova Scotia, Canada. *Journal of Pollination Ecology, 12,* 120-128. https://doi.org/10.26786/1920-7603 (2014)11

Sheffield, C.S., Westby, S.M., Smith, R.F., Kevan, P.G. (2008). Potential of bigleaf lupine for building and sustaining *Osmia lignaria* populations for pollination of apple. *The Canadian Entomologist, 140*(5), 589-599. https://doi.org/10.4039/n08-011

Shivanna, K.R., & Tandon, R. (2014). Fruit and Seed Biology. *Reproductive Ecology of Flowering Plants: A Manual*, 125-133. https://doi.org/10.1007/978-81-322-2003-9_10

Stern, R.A., Eisikowitch, D., Dag, A. (2001). Sequential introduction of honeybee colonies and doubling their density increases cross-pollination, fruit-set and yield in 'Red Delicious' apple. *Journal of Horticultural Science and Biotechnology, 76*, 17-23. https://doi.org/10.1080/14620316.2001.11511320

Tasei, J.N., & Picart, M. (1973). Observations sur le developpement d'*Osmia cornuta* Latr. et *Osmia rufa* L. (Hymenoptera Megachilidae). *Apidologie, 4*(4), 295-315.

Thamaraikannan, M., Palaniappan, G., Sengottuval, C. (2010). Can Indian Apples Beat Imports in Quality and Price? *World, 4*(69.587.24), 14-32.

Thomson, J.D., & Goodell, K. (2001). Pollen removal and deposition by honeybee and bumblebee visitors to apple and almond flowers. *Journal of Applied Ecology, 38*, 1032-1044. https://www.jstor.org/stable/827241

Vicens, N., & Bosch, J. (2000). Pollinating efficacy of *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae, Apidae) on 'red Delicious' apple. *Environmental Entomology, 29*(2), 235-240. https://doi.org/10.1093/ee/29.2.235

Volz, R.K., Tustin, D.S., Ferguson, I.B. (1996). Pollination effects on fruit mineral composition, seeds and cropping characteristics of 'Braeburn'apple trees. *Scientia horticulturae, 66*(3-4), 169-180. https://doi.org/10.1016/S0304-4238(96)00934-X

Wójtowski, F. (1979). Spostrzeżenia nad biologią i możliwościami użytkowania pszczoły murarki *Osmia rufa* L. (Apoidea; Megachilidae). *Roczniki Akademii Rolniczej w Poznaniu, 111*, 103-108.