



Effect of *Osmia bicornis* supplemental pollination on seed yield of forest seed orchards

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Received 17 October 2022 – Revised 8 March 2023 – Accepted 3 May 2023

Abstract – Our study investigated the possibility of using the red mason bee *Osmia bicornis* as a pollinator of forest seed orchards of frequently grown European trees: insect-pollinated *Tilia cordata* and *Prunus avium*. Moreover, we checked whether the presence of *O. bicornis* has an impact on the pollination results of *Quercus petraea*, which is wind-pollinated. Data were collected from 8 forest seed orchards in western Poland between 2018 and 2020. We assessed whether the presence of *O. bicornis* populations affects the number and mass of seeds and seed quality. Supplementing forest orchards with *O. bicornis* affected seed yield. The generalized linear mixed models indicated that the number of seeds and the proportion of viable seeds, but not seed mass, were higher in the *Tilia cordata* trees, when additionally pollinated with *O. bicornis* added as a pollinator.

red mason bee / forest management / *Tilia cordata* / *Prunus avium* / *Quercus petraea*

1. INTRODUCTION

In forestry, seed orchards are plantations of high-quality seeds of known origin (Kowalczyk et al. 2011), capable of germinating and furthering forest production (Chałupka et al. 2011). Seed orchards are created to obtain an abundant harvest of seeds with increased genetic value and to facilitate seed harvesting (Kocięcki 1965; Kowalczyk et al. 2011). Moreover, seed orchards constitute a valuable base for protecting the genetic variability of forest trees and the production of seed that is well adapted to specific environments (Nanson 1972; Kowalczyk et al. 2011). Due to the growing demand for seeds

of various species of trees over the last several decades, the creation of new seed plantations has been observed (Ruotsalainen 2014; Gömöry et al. 2020; Liesebach et al. 2021).

Wild bee species, the main group that provides the ecosystem service of pollination (Garibaldi et al. 2011, 2013), have been declining in many parts of the world (e.g., Potts et al. 2010; Goulson et al. 2015). Insect pollination services are worth 14.6 billion EUR annually in the EU and more than 24 billion USD in the USA (Leonhardt et al. 2013). Global pollinator decline and lack of their services have a significant negative economic effect on crop production worldwide (Kevan and Viana 2003). The observed decline in the populations of pollinators is noticeable in horticulture and agriculture (Kearns et al. 1998; Klein et al. 2007; Radmacher and Strohm 2010; Bąk and Wilde 2003). Moreover, the yield

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Manuscript editor: Peter Rosenkranz

from some seed orchards has also been low, and insufficient pollination has been suggested as a possible cause (Moncur et al. 1995). Commercially managed species such as honey bees, *Apis mellifera* (Apidae), are used to supplement wild bees to prevent low yields of various crops (Allsopp et al. 2008; Breeze et al. 2011; Garibaldi et al. 2013). Honey bees were also introduced in forest seed orchards of insect-pollinated trees such as *Eucalyptus* and *Acacia* to increase seed quantity and quality (Hardi and Kanso 1995; Moncur et al. 1995). Managed solitary bees of the genus *Osmia* also proved to be effective pollinators in several crops (Batra 1978; Torchio and Asensio 1985; Bosch and Kemp 2002). *Osmia bicornis* (Linnaeus 1758) (previously *Osmia rufa*) has been used as an additional pollinator in European fruit tree orchards and on other crops such as strawberries and oilseed rape from the 1970s (Hansted et al. 2014; Sedivy and Dorn 2014; Wilkaniec and Radajewska 1997; Gruber et al. 2011). However, the use of managed solitary bees as supplemental crop pollinators is still limited.

O. bicornis is a widely distributed univoltine, solitary, polylectic species native to Europe (Kronic and Stanisavljevic 2006). *O. bicornis* nest gregariously in artificial nesting materials. It is active from mid-April until the end of June in Central Europe (Steffan-Dewenter and Schiele 2008), but commercial rearing techniques ensure the availability of adults for release at a desired time of the year to synchronize bee emergence and activity with the bloom of a target crop (Bosch and Blas 1994; Kronic et al. 1995). The use of managed bees to supplement pollination positively affects the yield and quality of fruits (e.g., strawberries, fruit trees) compared to pollination by wild pollinators alone (Lye et al. 2011; Ryder et al. 2020). However, there is a lack of information on managed *O. bicornis* in forest orchard production systems. The role of *O. bicornis* in forest orchards has yet to be quantified, and managed wild bees are not commonly incorporated into forest orchard management. Therefore, research is required to support the

optimization of pollination services in the forest orchard. This study investigates the hypothesis that supplementing pollination by managed *O. bicornis* will increase the yield and the seed quality of forest orchards.

This study aimed to investigate the possibility of using *O. bicornis* as a pollinator of forest seed orchards of frequently grown European insect-pollinated trees: *Tilia cordata* and *Prunus avium*. Moreover, we checked whether the presence of *O. bicornis* has an impact on the pollination results of *Quercus petraea*, which is wind-pollinated. The following questions were addressed: (1) does the presence of *O. bicornis* populations in a seed orchard increase the number and mass of seeds? and (2) does *O. bicornis* pollination also affect seed quality?

2. MATERIALS AND METHODS

2.1. Study area

Small-leaved lime (a.k.a. linden) (*Tilia cordata* Mill.), sessile oak (*Quercus petraea* (Matt.) Liebl.), and wild cherry (*Prunus avium* L.) seed orchards located in western Poland were selected for the research. *T. cordata* was cultivated in 4 forest districts: Jastrowie (53°39'N; 116°74'E), Pniewy (52°48'N; 16°26'E), Łopuchówko (52°57'N; 17°09'E), and Świerczyna (53°42'N; 16°21'E). The sizes of these orchards were as follows: 4.00, 4.32, 2.20, and 4.43 ha. *Q. petraea* cultivated areas were located in Tuczno (53°22'N; 16°09'E) and Złocieniec (53°45'N; 16°07'E) and covered the area of 7.89 and 4.76 ha. *P. avium* orchards were located in Łopuchówko (52°57'N; 17°10'E) and Świerczyna (53°46'N; 16°30'E) and had areas of 2.18 and 3.98 ha respectively. These seed orchards were established at different times: Jastrowie in 1987, Łopuchówko (Prunus in 2007, Tilia in 1996), Pniewy in 1996, Świerczyna (Prunus in 2010, Tilia in 2000), Tuczno in 2005, Złocieniec in 2005.

2.2. Study species

2.2.1. *Tilia cordata*

The range of *Tilia cordata* Mill. covers almost the whole continental part of Europe. Its distribution is suboceanic to subcontinental. It is most abundant from north-eastern France through central Germany and Poland to central Russia and northern Ukraine (Radoglou et al. 2009). Their importance as a significant part of forest biodiversity is expected to increase, especially in the changing environment, due to climate change (Radoglou et al. 2009; De Jaegere et al. 2016; Zhelev et al. 2020). Because of their esthetic and cultural value, lime trees are also often planted in urban areas of Central Europe as park trees and along avenues and roads (Weryszko-Chmielewska and Sadowka 2010). *Tilia cordata* flowers in July. Limes are principally insect-pollinated trees, though wind-pollination also occurs. Bees are the main visitors and pollinators, even if other generalist insects are also observed on *Tilia* trees (Anderson 1976; Pigott 1991). Flowers of *T. cordata* are regarded as nutritionally very attractive for bees (Jabłoński et al. 2000).

2.2.2. *Prunus avium*

Modern silvicultural and management regimes should ensure the forest ecosystem biodiversity and obtain valuable wood that could increase the income of forest owners. Wild cherry (*Prunus avium* L.) is a very valuable forest tree species and one of noble hardwoods. It is a mainly European species and served as the origin of all cultivated forms. It blooms from April to May. Flowers are pollinated mainly by honey bees and wild bees, and the trees are generally not self-fertile (Welk et al. 2016).

2.2.3. *Quercus petraea*

Quercus petraea (Matt.) Liebl. is a widespread species native to most of Europe, found

from Scandinavia to the Iberian Peninsula. The wood from oaks is hard and durable and has been valued for centuries. Dieback and decline in oak have been reported in Europe since the early 1900s and have been attributed to single or combined effects of various abiotic factors. *Q. petraea* co-occur at many sites as the main component of temperate deciduous mixed forests. It is also appreciated as a park and roadside tree. The sessile oak is monoecious and wind-pollinated. Although temperate wind-pollinated species are usually discounted as bee resources, there is evidence that bees collect such pollen (Saunders 2018), and some wind-pollinated trees may be good protein sources (Roulston and Cane 2000). *O. bicornis* representatives eagerly visit oak flowers and collect large quantities of pollen (Hallmen and van Leeuwen 1990). Therefore, we included plantations of this species in our research to check whether the presence of additionally introduced pollinators may impact the yield of these trees.

2.3. Data collection

Data were collected from the 8 forest seed orchards for 3 years each (2018–2020). Twenty-four (12 experimental and 12 control) trees of *Tilia cordata* and 12 (6 experimental and 6 control) *Prunus avium* trees and 12 (6 experimental and 6 control) *Quercus petraea* trees were selected. The experimental and paired control trees without *O. bicornis* were from one clone for genetic compatibility. Selected trees (experimental and control) were at least 500 m away from each other. They were the same age and size and had similar diameters at breast height (DBH). Sixteen artificial bee nests and cocoons with *Osmia bicornis* were placed next to every experimental tree. Artificial nests consisted of 60–70 reeds (*Phragmites australis*) of different diameters (5–8 mm) and 20 cm length placed in plastic tubes. The artificial nests were located in dry and sunny places, 100 cm above the ground (Appendix, Figure A1). Cocoons were removed from nest cells in the winter months and maintained in a cold room under conditions

of continuous darkness with the temperature of 4 °C. Four to five days before being moved to *Prunus* and *Quercus* experimental plots, cocoons were transferred to 20 °C. Placing the release boxes in the orchards was synchronized with the flowering of the trees. The 1000 *O. bicornis* cocoons were placed in each release box.

Seeds were collected from the selected experimental and control trees to determine the quantity and quality of the seeds. The lime seeds came from branches of similar length and number of lateral branches located at 3 height levels (1.5, 3, and 4.5 m). Samples were additionally collected from 4 different geographic directions for each of the levels, i.e., 12 times from each tree. For oak and cherry trees, all seeds from the trees were collected. After harvesting, seed samples were assessed using the cutting method commonly used to evaluate seed material in forestry (Załęski et al. 2000). The following seed parameters were determined: the weight of a thousand seeds, the number of seeds: empty, healthy, rotten, and the ability to germinate. The results concerning the number of obtained seeds and their weight and germination capacity were compared between the experimental and control trees. The experiment was carried out so that the trees of the corresponding clones, which were control trees in the first year of the study, were experimental in the next year, and vice versa, only changing the location of aggregations of bees.

The number of samples obtained for *Prunus* and *Quercus* was lower than planned due to the lack of fruit production due to spring frost damage and water deficits.

2.4. Statistical analysis

We assessed differences in the proportion of viable seeds, the number of seeds, and the mass of the thousand seeds between the experiment (supplemental pollination by *O. bicornis*) and control (without supplemental pollination by *O. bicornis*) groups separately for tree species using generalized linear mixed-effects models (GLMMs) with year and site as random

factors and restricted maximum-likelihood (REML) estimator. We assumed the Gaussian distribution of mass of a thousand seeds, negative binomial of the number of seeds, and beta distribution of the proportion of viable seeds. Additionally, we assessed differences in the proportion of empty seeds of *Tilia cordata* and the proportion of non-viable seeds for both tree species between the control and experimental group. Prior to analyses, the mass of the thousand seeds was logarithm-transformed (\log_{10}). The proportion of viable seeds was treated as the response variable after applying a small, linear transformation to eliminate zeroes and ones (Smithson and Verkuilen 2006). Because of the small sample size for *Prunus avium*, we used a non-parametric *U*-Mann–Whitney test to assess differences in the proportion of viable seeds, the mass of the thousand seeds, and the number of seeds between the experimental ($n = 4$) and control groups ($n = 4$).

Additionally, we calculated the standardized effect size (Cohen *d*) for our response variable for experimental and control groups, separately for tree species, calculated using the *eff_size()* function in the *emmeans* package (Lenth 2022). Standardized effect sizes were calculated by dividing pairwise differences in estimates by the SD of the experiments (with *Osmia* bee) and controls (without *Osmia* bee) providing the context for those differences. Zero Cohen *d* values signify no difference in response variables between experimental and control groups, while positive and negative *g* values indicate trends toward increased and decreased effect of bees on response variables respectively. Next, we visualized the predicted effect size with corresponding 95 % confidence intervals for tree species separately for response variables.

The analyses were carried out in R 4.0.2 (R Core Developmental Team 2020). GLMMs were carried out using the *lme4* (Gaussian and negative binomial distribution) and *glmmTMB* (bet a distribution) packages (Bates et al. 2015; Brooks et al. 2017), and the data visualization using the *ggplot2* package (Wickham 2009).

3. RESULTS

All *O. bicornis* emerged from the cocoons exposed in *Prunus* and *Quercus* forest seed orchards in the spring, while in the summer, single cocoons remained unemerged in *Tilia* orchards. *O. bicornis* individuals nested in prepared artificial nests. They were also observed on experimental trees.

The generalized linear mixed models indicated that the proportion of viable seeds ($p=0.020$) and the number of seeds ($p=0.009$) in *Tilia cordata* significantly differed between the experimental and control groups (Figure 1). The number of seeds and the proportion of viable seeds were higher for trees additionally pollinated by *O. bicornis*. For the rest tree species, we did not find any significant differences in tested response variables ($p>0.05$; Table 1).

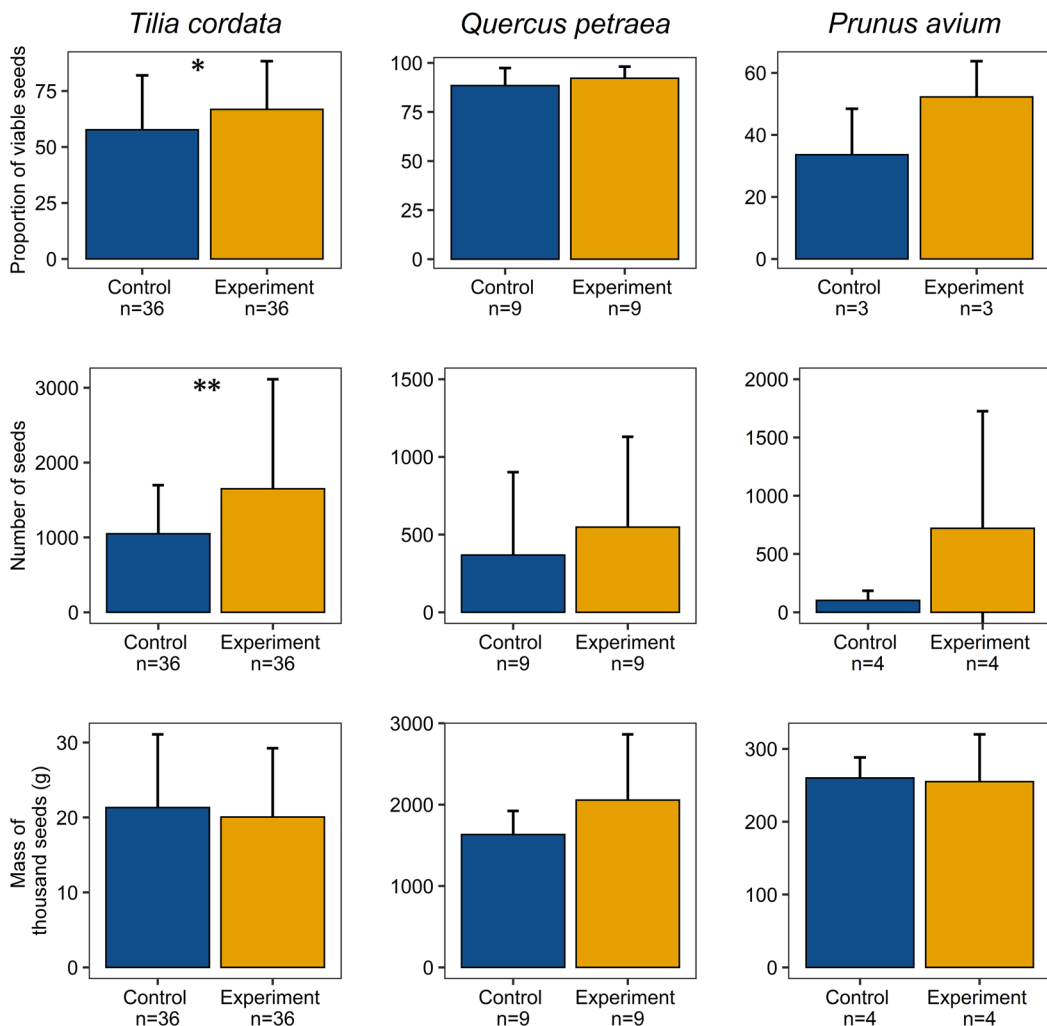


Figure 1. Mean (whiskers, SE) of the proportion of viable seeds, number of seeds, and mass of thousand seeds (g) for control (without supplementing pollination by *O. bicornis*) and experiment (with supplementing pollination by *O. bicornis*) trees of *Tilia cordata*, *Quercus petraea*, and *Prunus avium*. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$), n = number of trees sampled.

Table 1 The differences in the proportion of viable seeds, the number of seeds, and the mass of the thousand seeds between the experimental and control groups

Response variable	<i>Tilia cordata</i>			<i>Quercus petraea</i>			<i>Prunus avium</i>				
	*Statistic test	df	p	Effect size (95% CI)	*Statistic test	df	p	Effect size (95% CI)	*Statistic test	p	Effect size (95% CI)
Proportion of viable seeds	5.40	1	0.020	0.40 (-0.07; 0.86)	2.27	1	0.132	0.48 (-0.46; 1.42)	0.13	0.200	1.40 (-0.51; 3.20)
**Site	0.05 ± 0.22				0.00 ± 0.00						
**Year	0.53 ± 0.73				0.53 ± 0.73						
Number of seeds	6.74	1	0.009	0.35 (0.07; 0.62)	2.10	1	0.157	0.52 (-0.21; 1.27)	1.73	0.114	0.87 (-0.63; 2.30)
**Site	0.02 ± 0.13				0.62 ± 0.79						
**Year	0.15 ± 0.38				1.25 ± 0.11						
Mass of thousand seeds (g)	0.69	1	0.411	-0.19 (-1.07; 0.69)	2.59	1	0.129	0.76 (-1.97; 3.49)	0.44	0.686	-0.11 (-1.50; 1.28)
**Site	0.00 ± 0.05				0.00 ± 0.00						
**Year	0.03 ± 0.16				0.00 ± 0.00						

Values in bold indicate statistically significant differences

*Statistic test from mixed -effect models for *Tilia cordata* and *Quercus petraea* represented by *F* for the mass of the thousand seeds, Wald chi - square otherwise; statistic test for *Prunus avium* represent by *z*-value of Mann-Whitney *U*-tests

**Results of variance and SD for random effects are given

In Figure 2, we present the effect size (Cohen *d*) with corresponding 95 % confidence intervals for each tree species separately for tested response variables. The introduction of *O. bicornis* to forest seed orchards has a positive effect on the number of seeds and the proportion of viable seeds but the effects were not significant.

4. DISCUSSION

In this study, significant differences between treatments in two of three key characteristics of tree seed production were found. The number of

seeds and the proportion of viable seeds were higher in the *Tilia cordata* trees where pollinating insects were supplemented with commercially reared *Osmia bicornis*. No differences emerged across pollination treatments on *Quercus petraea* and *Prunus avium*. Additional analyses showed that the introduction of bees to forest seed orchards has a positive effect on the number of seeds and the proportion of viable seeds.

Insect pollinators are a crucial component in providing vital ecosystem services to crops and wild plants, and the lack of pollinators frequently limits seed production. The decrease in

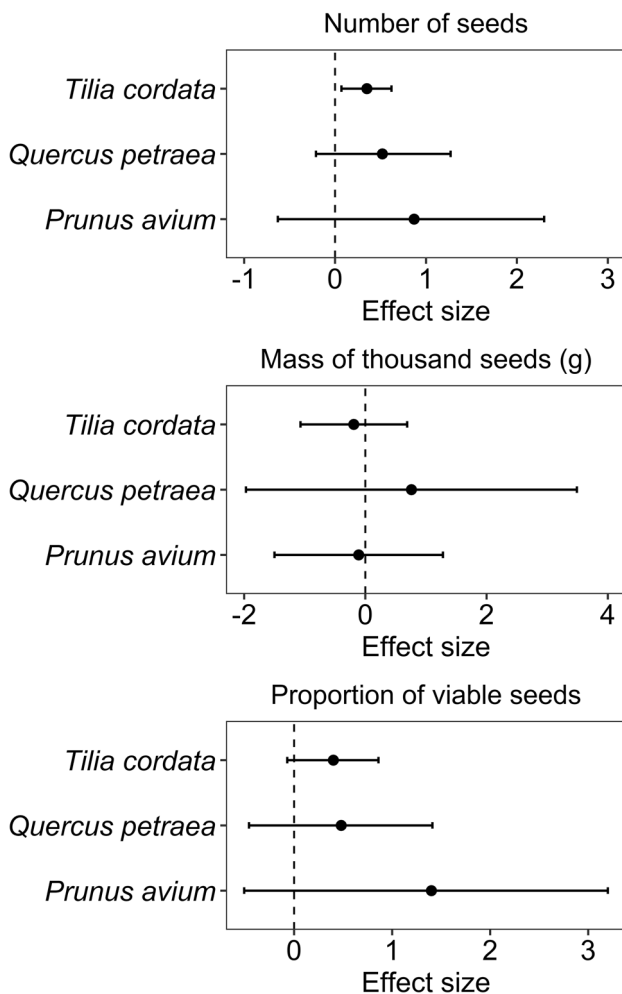


Figure 2. Effect size of supplementing pollination by *O. bicornis* on number of seeds, mass of thousand seeds (g), and proportion of viable seeds. The points represent mean effect sizes and 95 % confidence intervals.

the number of pollinating insects causes crop production to be increasingly dependent on managed pollinators. Only a few works is there information on the use of honey bees in the seed production of forest tree species (Loneragan 1979; Moncur et al. 1995; Hardi and Kanso 1995). Implementation of a native bee commercially developed and implemented for pollination can increase yield to meet the rising demand for forest products. To the best of our knowledge, this is the first field study in which the impact of solitary bee implementation in forest tree seed orchards has been studied for their effects on the seed yield. *O. bicornis* is widely distributed across Europe and is commonly used for commercial agriculture. Our research demonstrated that the red mason bee also has the potential as a forest orchard pollinator. It is thus possible to increase pollination and seed production with *O. bicornis* populations.

Moreover, because of its relatively short foraging range, *O. bicornis* would probably limit the pollination with pollen from outside the seed orchard compared to other commercial pollinators. Seed plantations are a particular type of forest cultivation established to produce seeds of high utility value used for afforestation (Kess and El-kassaby 2015). Pollen from individuals growing outside this area is considered contamination. Breeding procedures are limited to isolating seed plantations from potential sources of foreign pollen as effectively as possible (Adams and Burczyk 2000). With abundant food, *O. bicornis* bee flies 100–200 m from the nest. It is a generalist pollen forager collecting pollen from many plant families (Haider et al. 2013). However, their foraging behavior often shows a relatively high pollen constancy as these bees tend to prefer specific pollen sources when they are abundant near bee nesting sites (Radmacher and Strohm 2010; Sedivy and Dorn 2014). *O. bicornis* females specialize temporarily on plant species with high pollen availability to maximize pollen collection mass over time (Radmacher and Strohm 2010). This trait is advantageous for pollination in forest seed orchards. Moreover, *Osmia* bees forage at lower temperatures and in less favorable weather conditions and, therefore,

for a more extended period per day than honey bees (Vicens and Bosch 2000). This feature is valuable for pollinating early blooming tree species such as cherry. Ryder et al. (2020) indicated that *Prunus avium* fruits from orchards with supplemented pollination by *Osmia bicornis* were larger and heavier at harvest than those produced with wild pollinators alone. An increase in fruit quality has been reported in cherry orchards when *O. bicornis* contributes to pollination (Kuhn and Ambrose 1984; Wilkaniec and Radajewska 1997). Our research also indicated a positive effect of supplementing *O. bicornis* on *Prunus* cultivation. However, a too-small sample size influenced the obtained results.

A strong preference for oak pollen is consistent with many studies on *O. bicornis* (e.g., Ruddle et al. 2018; Šlachta et al. 2020; Bednarska et al. 2022). Oak is wind-pollinated and offers large quantities of pollen. Other researchers have also confirmed the increased preference of red mason bees for anemophilous plants (Wilkaniec and Radajewska 1997; Teper 2007). As a wind-pollinated plant, oak is not dependent on pollination by insects. However, in the case of many wind-pollinating plants, pollinating insects may impact their yield. In our research, the presence of *O. bicornis* did not adversely affect the yielding of oak trees. We observed a slight trend of increasing the number and mass of seeds, but the results were not significant.

4.1. Implications on seed orchard management

The results of this study can be used for improved seed production of forest orchards. Our study indicates the potential use of the managed species of the wild bee as a complementary pollinator in seed orchards. Seed production in a seed orchard can be increased by supplemental mass pollination by red mason bees, thanks to an increase in the number of seeds and the proportion of viable seeds.

The food resources of forest environments are relatively low. Therefore, the bee density in forest environments is lower than in open habitats

(Cierzniak 2003). This can pose a problem in the availability of an adequate number of pollinators in forest seed orchards that require pollinating insects. Low numbers of *Apis mellifera* and naturally occurring wild bees were recorded during the flowering period of examined forest seed orchards (unpublished data). That is why using managed species of solitary bees may be beneficial. High pollination efficiency, easy handling of the nests, and the lack of aggressiveness make it worth using *Osmia bicornis* as an alternative to honey bees. Nesting in large aggregations of these solitary bees and the possibility of using various artificial nesting materials are conducive to successful mass breeding. Moreover, the emergence of the adult can be manipulated to the flowering period of trees in forest orchards. Furthermore, managed honey bee colonies often suffer from increased prevalence of *Varroa destructor* parasites and viral, bacterial, and fungal diseases (Genersch 2010; Geslin et al. 2017), which, in turn, increases the costs of managing the hives, which takes both time and money. In the case of *Osmia bicornis* breeding, these problems are limited.

However, comprehensive and sustainable management should aim not only to improve pollination efficiency but also to maintain stable bee populations in the environment of forest seed orchards. That is why further research should also investigate the reproduction of *O. bicornis* in forest orchards to assess the costs and benefits of its commercial use and allow comparisons with alternatively managed pollinators. Also, more study is needed to make recommendations for ensuring the maintenance of supplementing pollination services by *Osmia bicornis* in forest ecosystems. Another issue is the impact of managed *O. bicornis* on local populations. Concern has arisen that managed bees may negatively affect wild bees (e.g., through competition with wild bees and pathogen spillover). Research on this subject is focused on the most common and widely used managed bees, honey bees and bumble bees. In contrast, the information concerning other managed bees, including *O. bicornis*, is limited (Mallinger et al. 2017). Moreover,

other possible solutions for increasing pollinator populations in forest orchards require consideration. Trees in plantations provide a rich source of food, but for a limited time. For this reason, additional wildflower plantings can furnish wild bees with nectar and pollen throughout the season and ensure greater abundance and stability of pollinator populations, thus supporting forest seed orchard pollination. Moreover, decreasing pesticide use or increasing nesting sites can also be beneficial.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1007/s13592-023-01013-z>.

AUTHOR CONTRIBUTION

MK: methodology, investigation, writing – original draft. WBC: conceptualization, writing – original draft. ŁD: statistical analysis, writing – review and editing. MF: conceptualization, methodology, supervision.

FUNDING

Research conducted by the statutory funding No. 506-511-04-00 of the Faculty of Veterinary Medicine and Animal Science Poznań University of Life Sciences, Poland; Department of Zoology.

DATA AVAILABILITY

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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