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RESEARCH ARTICLE

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A New Type of Bee Pollen from The Nests of Wild Bees (*Osmia rufa L.* syn. *O. bicornis*) in Forest Seed Orchards

Mateusz Kesy*
Department of Apidology, Poznan University of Life Sciences, Poznan, Poland

ABSTRACT

Seed orchards are used in forestry to avoid monoculture management in forest areas and also constitute a gene bank for various tree species. Today, several hundred seed orchards are located in the country in order to meet seed needs, preserve species diversity and preserve the genetic resources of forest management in the country. The paper presents an innovative solution to increase tree yield by introducing nests with solitary bees *Osmia rufa L.* (*syn. Bicornis*) into forest monocultures. During scientific research, significant amounts of bee pollen were observed that were not consumed by bee larvae. It was decided to observe the reasons for this and to determine the possibilities of using this type of pollen as a new bee product (not from honey bees *Apis mellifera L.*, which provides most of the known bee products). The aim of the work was to present the composition of a new type of pollen and show the possibilities of its use.

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Introduction

Bees are of great importance to humans and all nature, especially forestry [1-7]. Pollinating insects are a very important species for pollinating plants in orchards, plantations and forest crops, especially in the field of seed formation and bee products such as honey, pollen and propolis are used to support the treatment of many diseases [3,5,8-10]. Honey contains a rich chemical composition that determines its nutritional and medicinal properties, which is why it is considered to have healing and preventive properties. Bee products are also a source of antioxidants, and their quality depends on the climate, environment, soil and storage method [11]. Unfortunately, in recent years there has been an increase in the mortality of these beneficial insects, which is often related to the negative impact of humans on the environment [3]. However, the number of bee products available in stores is still large and consumers are interested in these products. The second very important bee product that bees provide us with is bee pollen (pollen grains), which is collected and most often brought by honey bees to the hive and in wild bees, it is most often collected and attached to the bee's belly and then, in smaller parts, it is brought to the bee nest made of reeds or other plant parts [11-13]. One of the most popular species of wild bees is *Osmia rufa L.*, which is commonly used in fruit and seed farming to increase the yield of various species of fruit trees and shrubs and is an important element of the plant pollination process [13]. This species has been well understood in terms of their biology, development and use, but various possibilities of using this bee in practice in various environments are still being learned [13-15]. This paper shows the results of research on an attempt to introduce this species into forest seed orchards, where this bee has proven to be an effective pollinator and where it can be an important complementary element in seed production [16-18]. Bees in every work environment moisten the collected bee pollen with nectar or honey from the hive, which is why it has a characteristic taste and smell [12,19]. In honey bees, pollen grains have a similar shape, size and weight while in bees *Osmia rufa L.* The pollen may vary in weight and shape because the bees make many flights to gather food to feed their larvae, collecting this pollen in various places, sometimes different in size, which sometimes creates restrictions for these insects [11]. To collect one collection of pollen, honey bees must visit from 7 to 120 flowers and it depends on the type of plant from which the bees are currently collecting food [11], while in Osmia bees, the number of visits to flowers and flights for food is probably higher because the supply of pollen in the nests is several times larger than the pollen deposits of honey bees (the author's own observations). Bees bring pollen from various plants to their nests or hives, so it may also differ in color, which results, among other things, from the type of plant and the fact what the bees moistened the

Contact: Mateusz Kesy, E-mail: matkesy@gmail.com

collected pollen with [11,20]. It is known that honey bees can use honey for this purpose and *Osmia rufa* bees probably use water and mandibular secretions [11]. The most common pollen grains are yellow and orange, but there are also greenish (from pear pollen), cream-gray (from raspberry), brown (from clover), red-orange (from reseda pollen), dark red (from chestnut), dark blue (from echium) and dark gray or almost black (from poppy pollen). Pollen is the basic protein food for bees, which determines the proper development of their families and populations in wild bees [13]. The oldest records regarding the use of bee pollen in medicine date back to ancient times, where it was attributed properties essential to life and was called "life-giving powder" or "ambrosia" [11]. In the middle Ages, pollen was used as a sedative. Many valuable nutritional and therapeutic properties are currently attributed to flower pollen, which result from its exceptionally rich chemical composition. The most important biological properties of pollen are: Nutritional, regenerative, hypolipemic, detoxifying, adaptogenic, antibacterial, anti-inflammatory and anticancer properties. Despite its various properties, pollen may contain fungi and other microorganisms that bees collect with the pollen [21]. Given the important role that bee pollen plays in medicine and other fields of science [22]. In this study, the author decided to examine the composition of bee pollen collected by *Osmia rufa L.* bees and to determine the possible future use of this product by humans. So far, bee pollen is obtained from honey bees and is easily taken from them when they arrive at the hive, however, in the case of wild bees, collecting pollen is more difficult because access to the interior of the bees' nests is difficult (plant stems are usually a dozen or several dozen centimeters long and inside the bees create separate nests for each of the bee larvae, separated by a partition made of, among others, clay or another durable substance collected from the bees) by bees from the immediate environment [13,14,19]. Bee pollen from wild bees can be obtained most easily and quickly when full larval development is completed, a few weeks after the bee closes the last brood chamber. The bees' nests are then opened to clean nests from parasites and undeveloped and dead larvae. At this point, it is also possible to remove bee pollen from the nests that has not been eaten by the bees. Such pollen is usually found in bee nests for several to several weeks from the moment the bee delivers the pollen to the nest, therefore, it often contains many microorganisms (this phenomenon has been well described in the author's other manuscript [15]. This study collected data on bee pollen as an unknown bee product originating from forest seed plantations (Figure 1) where scientific research was conducted on the possibility of using bees *Osmia rufa L.* as a factor increasing the yield of trees of selected plant species (*Tilia cordata* Mill., *Quercus petraea* Liebl. and *Prunus avium L.*). These bees have been used many times in various work environments and new possibilities for their use are constantly being discovered [14, 23-25].

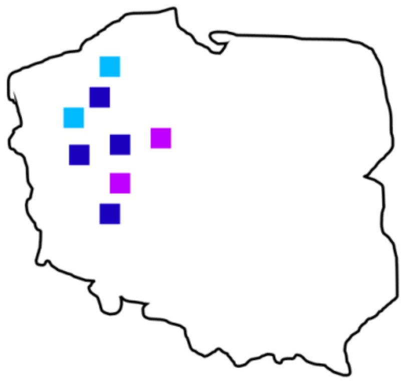

Figure 1. Distribution of seed orchards covered by the research (Poland). **NOTE:** (■)- *T.Cordata*; (■)- *Q.Petraea*; (■)- *P.Avium*.

Due to the initiated cooperation between specialists of this unit in the field of zootechnics, the need to conduct interdisciplinary research on the rearing of solitary bees in selected forest biotopes was identified.

Materials and Methods

Nests with mason bee imago (500 females and 500 males) were placed in 2018 and 2019 on seed orchards: 4 small-leaved linden (*Tilia cordata* Mill.), 2 sessile oak (*Quercus petraea* Liebl.) and 2 bird cherry (*Prunus avium*

L.) these crops had a varied area (2-7 ha). The bee rearing method was used, described, among others, in the work of Wójtowski et al [25]. The research space consisted of forest seed orchards, i.e. appropriately selected plant clones (groups of individuals with the same genetic composition, obtained from one individual through asexual reproduction) or families (offspring of the mother tree created through sexual reproduction) subject to management and isolation [26]. Home of bees witch imago was installed in each plot, containing 16 nests with reed stalks (*Phragmites australis* (Cav.) Trin. ex Steud) 18 cm long and about 6-9 mm in diameter, about 45 pieces for each place, along with the bees. The nests were always positioned so that the opening to the reed stalks faced south. Home of bees were placed in a similar way on each seed orchards, i.e. at the edge of the crops, about 5 meters from the fence, adjacent to other forest or agricultural crops through the fence. The location of the nests was determined by the need to maintain a distance of approximately 300 meters between the experimental and control trees (maximum flight range of red mason bees) in order to conduct the experiment. The exposure of bee cocoons was related to the flowering date of crops, which was closely related to weather conditions (nests were exposed to crops when the daytime temperature was around 15˚C) and differed slightly in both seasons. For bird cherry crops this occurred between April 10-20, for sessile oak between May 20-30 and for small-leaf linden between June 24 and July 4. The paper also used materials regarding the establishment and maintenance of seed orchards from Forest Districts. Materials were obtained electronically and in person.

In October, an analysis of nests was carried out, assessing the total number of chambers built by the mason bee, parasitized chambers and those in which dead larvae and bee pollen were found, as well as the number of imago pollen obtained. Additionally, the pollen collected by the mason bee in the rearing chambers was assessed, determining the food base of bees on individual seed orchards. For this purpose, the "per cell" method was used, which involved assessing the frequency and share of pollen of individual taxa, calculated from a minimum of three pollen cells. It determines the share of pollen within four groups: Dominant >45%, accompanying 16%-45%, single 3%-16%. Occasional <3%.

RESULT AND DISCUSSION

The manuscript contains information about bee rearing on different forest seed orchards (Tables 1-3) (Figures 2-4) and bee pollen obtained in this way (Tables 4-5). This product has not yet been recognized in terms of its possible applications, but the research results below show the first information about this product and the possibilities of obtaining it on a larger scale.

Table 1. Results regarding the reproduction of *Osmia rufa L.* in the forest seed orchards (*Tilia cordata* Mill.).

Area	Year	Total number of nesting sites constructed	Number of nests with parasites	Number of nests with dead larvae and pollen	Number of empty nests	Number of nests with adult healthy insects
Świerczyna	2018	2656	49	464	86	2067
	2019	28	20	6	0	2
Jastrowie	2018	1455	222	208	94	925
	2019	132	80	9	0	43
Pniewy	2018	1166	0	329	16	828
	2019	26	22	2	0	2
Łopuchówko	2018	2135	192	875	9	1001
	2019	15	8	0	0	7

Table 2. Results regarding the reproduction of *Osmia rufa L.* in the forest seed orchards (*Quercus petraea* Liebl.).

Area	Year	Total number of nesting sites constructed	Number of nests with parasites	Number of nests with dead larvae and pollen	Number of empty nests	Number of nests with adult, healthy insects
Tuczno	2018	3273	2	848	14	2393
	2019	1401	290	460	0	640
Złocieniec	2018	5212	68	657	11	4478
	2019	1330	210	520	20	570

Table 3. Results regarding the reproduction of *Osmia rufa L.* in the forest seed orchards (*Prunus avium L.*).

Area	Year	Total number of nesting sites constructed	Number of nests with parasites	Number of nests with dead larvae and pollen	Number of empty nests	Number of nests with adult, healthy insects
Świerczyna	2018	3828	0	586	103	3139
	2019	630	20	160	0	450
Łopuchówko	2018	4455	0	567	66	3822
	2019	2700	40	550	0	2110

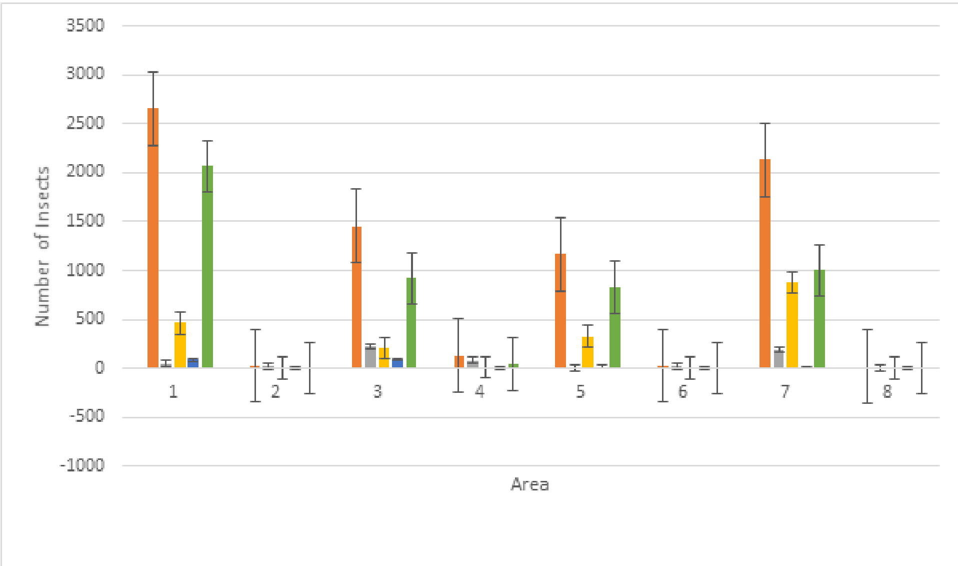

Figure 2. Results regarding the reproduction of *Osmia rufa L.* in the forest seed orchards (*Tilia cordata* Mill.). **NOTE:** ( )A; ( )B; ( )C; ( )D; ( )E.

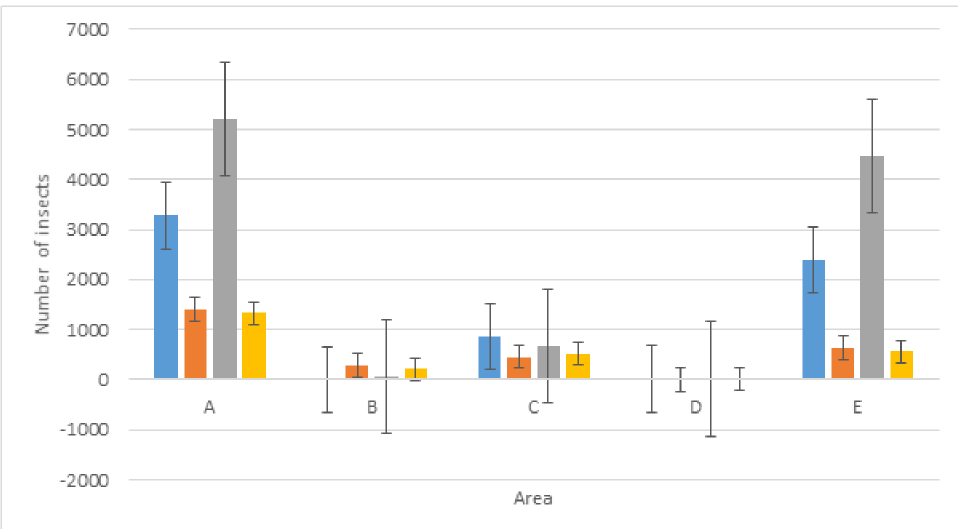

Figure 3. Results regarding the reproduction of *Osmia rufa L.* in the forest seed orchards (*Quercus petraea* Liebl.).

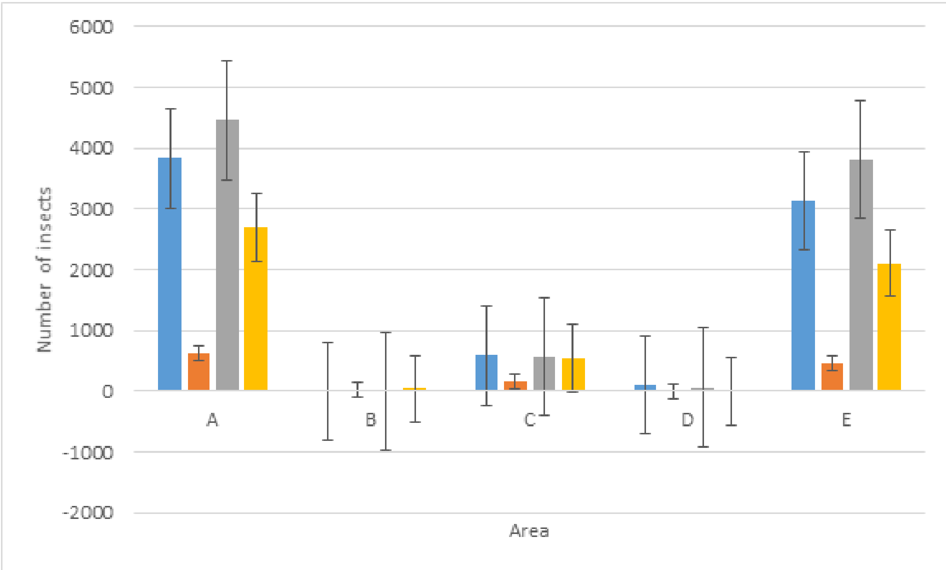

Figure 4. Results regarding the reproduction of *Osmia rufa L.* in the forest seed orchards (*Prunus avium L.*).

Table 4. The share of individual tree and shrub species around seed plantations (Based on data from the mobile forest data bank (Bank Danych o Lasach (lasy.gov.pl) access 27.02.2022.

Seed orchards	Species of forest trees on seed orchards (<1 km)
Tilia cordata Mill.	BK, CZM, DB, DG, GB, GŁG, JD, JW, LP, MD, SO, SW, TP, WZ AK, BRZ, CZM, DB.S, DG, JW, LP, MD, SO, SW
Quercus petraea Liebl.	BK, BEZ.C, BRZ, CZM, DB, JRZ, KL, OS, SO, SW
Prunus avium L.	BK, BRZ, CZM, DB, DB.S, GB, MD, OS, SO, SW
Abbreviations: AK: *Acacia*; BK- *Fagus sylvatica*, BRZ- *Betula*, DB.S- *Quercus robur*, DB.B- *Quercus petraea*, DBC- *Quercus rubra*, DG- *Pseudotsuga*, GB- *Carpinus betulus*, JD- *Abies*, JS- *Fraxinus*, JW- *Acer pseudoplatanus*, KL- *Acer*, LP- *Tilia cordata*, MD- *Larix*, OL- *Alnus*, OS- *Populus tremula*, SO- *Pinus*, SW- *Picea abies*, TP- *Populus*, WB- *Salix*, WZ- *Ulmus*, JRZ- *Sorbus*, CZM- *Prunus*, LSZ- *Corylus*, Bez.C- *Sambucus nigra*	

Table 5. Types of pollen found from nests *Osmia rufa L.*

Seed orchards	Types of pollen found from nests Osmia rufa L.
Tilia cordata Mill.	Tilia (87%*), others: Brassica napus, Frangula, Fagus, Rumex, Trifolium, Plantago, Pinus, Caryophyllaceae, Anthriscus, Urtica
Quercus petraea Liebl.	Quercus (28%*), others: Brassica napus, Prunus, Ulmus, Ranunculaceae, Lamiaceae, Pinus, Taraxacum, Ranunculaceae, Cytisus, Rubus, Senecio, Frangula, Ribes, Malus, Acer
Prunus avium L.	Prunus (1%*), others: Quercus, Pinus, Aesculus, Fraxinus, Rubus, Brassicaceae, Fagus, Juglans, Carex, Acer, Salix, Taraxacum, Ciutisus, Brassica, Frangula
Note: The table shows the maximum highest values (%) that were recorded during the analyses.	

In the forest seed orchards of *Tilia cordata* Mill, it was noticed that wild bee populations developed best in the first research season. The following year, bee rearing deteriorated due to unfavorable weather during the bees' flights and work. Reproduction, i.e. the duplication of the initial number of bee individuals, was the highest in the seed orchards in and the lowest.

A-Total number of nesting sites constructed, B- Number of nests with parasites, C- Number of nests with dead larvae and pollen, D- Number of empty nests, E- Number of nests with adult, healthy insects.

In the forest seed orchards of *Quercus petraea* Liebl., it was noticed that wild bee populations developed best in the first research season. The following year, bee rearing deteriorated due to little unfavorable weather during the bees' flights and work. Reproduction, i.e. the duplication of the initial number of bee individuals, was the highest in the seed orchards and the lowest in 2019 year in the same area. The work of bees in the second season was less exposed to high temperatures than in the case of *Tilia* crops.

A-Total number of nesting sites constructed, B- Number of nests with parasites, C-Number of nests with dead larvae and pollen, D- Number of empty nests, E- Number of nests with adult, healthy insects.

In the forest seed orchards of *Prunus avium L.*, it was noticed that wild bee populations developed best in the first research season (2018). The following year, bee rearing deteriorated due to little unfavorable weather during the bees' flights and work. Reproduction, i.e. the duplication of the initial number of bee individuals, was the highest in the seed orchards and the lowest in 2019 year in the same area. The work of bees in the second season was less exposed to high temperatures than in the case of *Quercus* and *Tilia* crops.

A-Total number of nesting sites constructed, B- Number of nests with parasites, C- Number of nests with dead larvae and pollen, D- Number of empty nests, E- Number of nests with adult, healthy insects.

The scientific research also determined the type of plants around bee nests located in forest areas [Tables 4-5]. Thus, the food base that bees could use was identified and compared with the type of pollen found in bees' nests.

In the forest environment covered by observations, the most abundant food for bees were *Tilia* crops. Less diverse trees and shrubs occurred within the flight range of *Osmia rufa* bees in the area of other seed crops.

Various types of *Osmia rufa* L. pollen have been identified in bee nests. The results are presented below.

Among the analyzed bee pollen samples, it was observed that insects consumed various foods on all seed orchards. Even though the dominant plants in each crop were: *Tilia*, *Quercus* and *Prunus*, insects sometimes avoided the flowers of these crops. The most pollen was collected by bees whose houses were placed on *Tilia* seed orchards in the monoculture area, and the least by the *Prunus* seed orchards. An interesting phenomenon is the fact that *Osmia rufa* bees use *Quercus* pollen because these plants are strongly wind-pollinated. However, at the stage of planning the experiment, the author decided that it is worth examining the scale of this phenomenon and determining the level of interest in *Quercus* pollen by these insects.

CONCLUSION

The results of field and laboratory observations show that bee pollen from wild bees is a very interesting example of a new bee product, which in the future may be used in various fields of science and perhaps previously unknown properties of this product may be discovered. The information presented in the work indicates the possibility of more extensive research on this product and provides information about the new discovery of a type of bee pollen. So far, the properties and uses of bee products derived from *Apis mellifera* honey bees have been quite well recognized, but information on the use of bee products derived from wild bees still seems to be very incomplete and selective. By analyzing information about the possibility of using the *Osmia rufa L.* species for pollination of forest seed crops, more comprehensive research on the properties and possibilities of using bee pollen can be planned in the future. Scientific work is planned to continue.

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