FOOD PLANTS OF *Megachile rotundata* L. DETERMINED BASED ON PALINOLOGICAL ANALYSIS OF FAECES

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S u m m a r y

In the years 2003-2004 samples of faeces of *Megachile rotundata* L. for palinological analysis were collected. These samples were collected for the whole time of the bee's flight period. Each day after dark, the colonies established in tubes were covered with a close-mesh isolator. The following morning, bees while trying to exit the nest, swarmed the isolator and excreted onto its surface. Microscopic preparations of collected faeces were made. The faeces were then subjected to a palinological analysis.

In the two years of research, a total of 56 microscopic preparations of *Megachile rotundata* faeces were made. Of these 56 preparations, 46 types of pollen belonging to 23 plant families were identified. In the first year of the experiment 39 types of pollen were identified. In the following year only 20 types. It was not possible to identify a small part of the pollen due to its degradation in the process of digestion. Damaged pollen grains belonged probably to plant species that produce grains of fine exine. These are the most susceptible for conditions in insects' digestive systems. On the basis of detailed palinological analysis of faeces for each year of the research, a food supply sequence of *Megachile rotundata* was made. It presents differences in plants species visited by bees over their forage season.

Keywords: *Megachile rotundata,* food plants, pollen analysis of faecese.

INTRODUCTION

The *Megachile rotundata* L. and *Osmia rufa* belong to the Megachilidae family. The common characteristic for these insects is that the pollen is collected on the stiff hairs of the abdominal sterna. Another common characteristic is their nesting in tunnels. The *Megachile rotunadata* bees overwinter in the prepupa stage. While leaving their cocoons in the spring they require warmer temperature levels to complete development. Their flight period starts in July. The period of foraging is much later in comparison to *Osmia rufa (Ruszkowski et al. 1998). Megachile rotunadata* requires a temperature of greater than 70°F/ 21°C (Hobbs 1967) for flying. The bees build nests in tunnels. Each cell is constructed from circular discs cut from plant leaves and petals using the bee's mandibles.

The *Megachile rotunadata* species is a very good pollinator of entomophillus *Medicago* crops. The *Meditago* plants form seeds when pollinated. Honey bees are less effective in pollinating these plants because of the specific flower structure (Jabłoński 1970 after Bohart 1948). In a flower of Fabaceae the two fused petals form a structure called the keel. While searching for nectar or pollen, the bee exerts pressure upon the keel petal. This pressure releases the sexual column, with its stigma and anthers. This structure then strikes the bottom of the bee's head. Honey bees become disinclined to visiting *Medicago* flowers after this structure strikes them on the bottom of their heads. The *Megichale rotunadata* can avoid the striking and often visits these flowers. In

one-minute the bee is able to visit and pollinate 10 *Medicago* flowers (Ruszkowski et al. 1985).

Gosek et al. (1992) stated that *Megachile rotunadata* often builds nests in holes drilled in hard sprouts of *Siringa*, *Corylus* and *Acer* or in *Tilia* wooden blocks. It also populates the tracing paper pipes. Those authors stated that the best effect of rearing this species is when the diameter of the pipe is 6-7 millimeters. Styrofoam elements with rows are also used in rearing *Megachile rotunadata*. The elements set together, alongside the rows, form blocks with holes where solitary bees nest.

The *Megachile rotunadata* was first used for pollination of alfalfa seed crops in the USA and Canada when the bees' mass rearing method was worked out (Bohart 1962, 1970; Stephen 1961, Hobbs 1964, 1967). Studies of the rearing methods of this species were conducted in Poland in Puławy by Ruszkowski's team (Ruszkowski et al. 1974, 1975, 1976, 1977, 1979). Afterwards Ruszkowski and Bilinski (1984a) started their research on the use of *Megachile rotunadata* for pollinating the field crops of *Medicago*. These authors studied the possibility of using this bee species for pollinating cucumber crops (Ruszkowski and Bilinski 1984b). Bilinski (1985) presented the usefulness of *Megachile rotunadata* in isolated crops and in greenhouses.

In the years 1961-1977 Ruszkowski et al. (1980) investigated plants visited by *Megachile* bees. They stated that *Megachile rotunadata* visit 40 plant species belonging to 13 plant families. Those plants mainly were of Fabaceae (35% of total), Asteraceae (17,5%) and Lamiaceae, Scrophulariaceae, Rosaceae and Brassicaceae (7,5% of each).

There are few known investigations based on palinological analysis of faeces as a method to determine food plants of insect species. In the middle of the XX century, Brian (1951) conducted palinological analyses of faeces of three species of bumblebee larvae. Other researches (Kozłowska i Warakomska 1984) analyzed mass dissected from the digestive system of insects belonging to the Syrphidae family. Palinological analysis of contents of the digestive system of *Coccinella septempunctata* proved that this predatory beetle feeds on pollen (Anasiewicz 1992). Palinological analysis of faeces was also used by Golding i Edmunds (2003). They used it as a method to determine the food plants of Diptera belonging to the Syrphidae family. These authors had also used this method as a means of avoiding the necessity to kill the insects. The same year Teper (2003) published results of preliminary observations of *Bombus terrestris* food plants based on palinological analysis of its faeces. The full results of the observation of *Bombus terrestris* and *Osmia rufa* food plants were published in the following years (Teper 2006, 2007).

The aim of the study was to verify whether the method of pollen analysis of faeces, as safer to insects and less labor consuming that observational, may be applicable in the determination of plants visited by *Megachile rotunadata*.

MATERIAL AND METHODS

In 2003 and 2004, colonies of solitary bees were localized at Górna Niwa in Puławy, Poland. Samples of faeces were collected from the 4th of June to 17th of August 2003 and between 6th of June and 6th of August 2004.

Collection of samples of *Megachile rotunadata* faeces

Due to the colonial character of the life of solitary bees, the method of collecting

faeces for the determination of *Osmia rufa* food plants was used (Teper 2007).

The *Megachile rotunadata* forages in the summer time. The bees overwinter in the prepupa stage. In the spring time the bees require a higher temperature level to complete its development. In laboratory conditions, when rearing bees for pollinating seasonal crops, bees emerge from cocoons when incubated at 30°C for 21 days.

In the experiment, a wooden chest was prepared. Tracing paper pipes with a diameter of 6 millimeters were put in holes drilled in the front wall of the chest. The pipes were plugged with plaster on one end. A cardboard box with emerging bees was placed under the wooden chest. In a few days time bees stared establishing the nest. Each evening the colony was covered with a close-mesh isolator on the side of the pipe outlets. In order to draw the isolator aside the pipes, small wooden sticks were attached to the chest corners. The bee colony was then covered until the following morning. Trying to leave the nest, the bees swarmed the isolator and excreted faeces out its surface. The *Megachile rotundata* is termophilic and starts foraging at temperatures above 20°C. In cooler and cloudy days the isolator was taken away at midday. These days samples of faeces were smaller depending on bee activity. The faeces-covered isolator was then taken and dried. The dry faeces were later crumbled away onto a paper and poured into tagged paper bags. Samples of faeces were collected daily over the entire flying period of bees (45 days in 2003 and 32 days in 2004).

Preparing microscopic preparations from *Megachile rotundata* faeces

At the end of the *Megachile rotundata* flying period, collected faeces stored in paper bags in the refrigerator, were poured out into pits of plates for spot tests with 1-2 drops of distilled water. The plates were left for a few hours for the soaking period. Afterwards, the contents were carefully stirred using a glass rod. Smears were performed in an inoculation loop onto tagged microscopic slides. The slightly dried preparations were covered with cover glasses with some glycerol gelatin.

Microscopic analysis of the collected material

Pollen grains of microscopic preparations were identified with the use of available pollen atlases (among others Sawyer 1981, 1988; Ricciardelli d`Albore 1998; Bucher et al. 2004) and own collection of over 300 reference preparations using Zander classification (1935, 1937, 1941, 1949, 1951). In each microscopic preparation pollen was determined, whenever possible, as to genus, species, family or type of build. Furthermore, pollen grains within the Brassiceae plant family were divided into small-Brassiceae (pollen grains below 20μm), Brassiceae (pollen grains between 20-30μm) or big-Brassiceae (pollen grains above 30μm).

RESULTS

Over the two experimental years, in a total of 56 microscopic preparations of *Megachile rotundata* faeces, the pollen grains of 23 plant species were determined. From the whole material 46 pollen types were identified: *Achillea* type, *Anthriscus* type, Asteraceae, Brassicaceae, small-Brassicaceae, big-Brassicaceae, Caryophyllaceae, *Centaurea cyanus*, *Centaurea jacea*, *Chenopodium*, *Cichorium* type, *Cirsium* type, *Convolvulus*, *Crocosmia*, *Echium*, *Euphorbia*, Fabaceae, *Fuchsia*, Poaceae, *Helianthus* type, *Heracleum* type, *Impatiens*, Lamiaceae, *Lonicera*, *Lythrum*, *Malus* type, *Medicago*, *Melilotus*, *Phacelia*, *Phaseolus*, *Phlox*, *Pinus*, Polygonaceae, Rosaceae, *Salvia*, *Solidago*

Table 1

Food supply sequence of *Megachile rotundata* based on pollen analysis of samples of faeces collected in 2003

No.	Type of pollen	July																										August													
		4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1.	*Heracleum* type																																											
2.	Poaceae																																											
3.	*Symphytum*																																											
4.	*Tilia*			→	→		→	→	→	→	→	→				→		→	→		→	→		→	→	→																		
5.	*Solidago* type			→	→		→	→	→	→	→					→		→							→				→	→	→	→			→		→			→	→			
6.	Brassicaceae			→	→		→	→	→	→	→					→		→		→	→				→	→			→	x	→	→	→			→	→			→	→	→	
7.	*Melilotus*			→	→		→	→	→	→	→					→		→							→						→					→		→			→	→	
8.	Caryophyllaceae			→	→		→	→	→	→	→	→		→		→																											
9.	Lamiaceae		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	
10.	*Anthriscus* type		→	→	→	→	→	→	→	→	→		→	→		→		→			→		→	→	→				→	→	→	→	→	→	→	→	→	→	→	→	→	→	
11.	*Vicia*			→	→		→	→	→	→	→				→	→		→		→				→	→	→			→	→	→	→	→	→	→	→	→	→	→	→	→	
12.	*Trifolium repens* s.l.			→	→	→	→	→	→	→	→				→	→		→							→	→	→	→	→	→	→	→	→	→		→	→	→			→	→	
13.	Fabaceae																																										
14.	*Convolvulus*			→	→	→	→	→	→	→	→	→	→																														
15.	*Medicago*																								→							→					→	→	→		→		→	→	
16.	*Phaseolus*						→	→	→	→	→					→		→				→			→		→					→					→	→			→	→		
17.	*Achillea* type						→	→	→	→	→					→	→	→			→	→			→				→			→		→		→	→						
18.	*Centaurea cyanus*						→	→	→	→	→	→	→	→	→	→	→	→	→	→	→		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→		→		
19.	*Trifolium pratense*						→	→	→	→	→	→				→	→	→		→	→	→			→	→	→	→	→			→	→	→	→					→	→	→	→	
20.	Asteraceae						→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	
21.	*Euphorbia*																																										
22.	*Chenopodium*																	→	→	→	→	→	→	→	→	→		→		→	→	→	→	→	→	→	→		→	→	→				
23.	*Cirsium* type																																										
24.	*Malus* type																																										
25.	*Helianthus* type																								→																		
26.	*Centaurea jacea*																								→	→	→	→	→	→													
27.	*Impatiens*																								→	→			→	→	→	→	→	→	→	→	→	→	→	→	→		
28.	*Viola*																																										
29.	*Crocosmia*																																										
30.	*Phlox*																																										
31.	Rosaceae																																						→		→		→	→	→	
32.	*Pinus*																																											
33.	*Zea mays*																																								→				
34.	*Ulex*																																												
35.	*Cichorium* type																																								→				
36.	*Phacelia*																																												
37.	Polygonaceae																																												
38.	*Salvia*																																												
39.	*Fuchsia*																																												
40	others																																												

→ - probable continuity of nectar flow, others – pollen grains not identified due to their considerable damage

Table 2

Food supply sequence of *Megachile rotundata* based on pollen analysis of samples of faeces collected in 2004.

No.	Type of pollen	July																									August					
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6
1.	*Melilotus*																				→		→	→	→				→				
2.	*Trifolium repens* s.l.			→				→	→		→		→		→				→	→	→	→	→	→	→	→							
3.	*Solidago* type							→													→		→	→	→				→				
4.	small-Brassicaceae		→					→	→																								
5.	big-Brassicaceae		→	→			→	→	→			→																					
6.	*Achillea* type																																
7.	*Lonicera*																																
8.	*Trifolium pratense*		→		→		→	→	→				→		→	→			→		→		→	→	→	→		→	→	→			
9.	*Anthriscus* type			→				→	→	→	→		→		→																		
10.	*Tilia*						→	→		→	→	→	→	→																			
11.	*Convolvulus*																																
12.	*Teucrium*						→	→																									
13.	*Echium*																																
14.	*Lythrum*						→	→	→	→	→																						
15.	*Vicia*							→																									
16.	*Salvia*																																
17.	Caryophyllaceae										→	→	→	→		→	→	→	→	→	→	→	→	→	→	→	→	→	→	→	→		
18.	*Medicago*												→								→	→	→	→	→				→	→	→		
19.	*Phacelia*																																
20.	*Taraxacum* type																																
21.	Others																																

→ - probable continuity of nectar flow others – pollen grains not identified due to their considerable damage

type, *Symphytum*, *Taraxacum* type, *Teucrium*, *Tilia*, *Trifolium pratense*, *Trifolium repens* s.l., *Ulex*, *Vicia*, *Viola tricolor*, *Zea mays*. In faeces collected in 2003, 39 types of pollen were identified (Table 1), whereas in 2004 only 20 types (Table 2). A small part of the pollen grains could not be determined due to considerable damage to its exine.

Detailed analysis of samples of *Megachile rotundata* faeces enabled the food supply sequence for each experimental year to be determined (Table 1 and 2) according to the method of Ruszkowski and Żak (1974).

In 2003 (Table 1) the first sample of faeces was collected on the 4th of July in which pollen of *Heracleum* type, Poaceae, *Symphytum*, *Tilia*, *Solidago* type, Brassicaceae, *Melilotus*, Caryophyllaceae, Lamiaceae and *Anthriscus* typ were identified. In the following days, various species of plants were visited by bees. It is important to note that *Megachile rotundata* preferred to visit *Solidago*, and also plants of the Brassicaceae family and *Melilotus,* over the whole foraging period. The bees often visited *Trifolium repens*, *Phaseolus*, plants of *Achillea* type and *Trifolium pratense*. After the 20th of July the *Megachile rotundata* also visited *Medicago*. The collection of samples of faeces was not possible for 12 days over the whole 45 days long experiment. This was due to rainy weather or temperature below 20°C. In these conditions solitary bees did not forage.

In 2004 (Table 2) sampling started on the 6th of July. Pollen of *Melilotus*, *Trifolium repens*, *Solidago*, plants from the Brassicaceae family, *Achillea*, *Lonicera* and *Trifolium pretense* were identified in collected samples. In the following days, the pollen grains of *Achillea* i *Lonicera* were not found, but *Tilia* and plants with pollen of *Anthriscus* type appeared. Also in the middle of July pollen of *Medicago* was identified. During the next few days the bees visited various species of plants. Over the whole sampling period, the pollen of *Melilotus*, *Solidago* and *Trifolium pratense* was identified. The *Medicago* plants were visited from the 16th of July until 6th of August in 2004. This was several days earlier compared to the first year of the research. The second year sampling ended on the 6th of August. The collection of samples of faeces was not possible for 5 days over the whole 32 day long experiment, due to weather conditions.

DISCUSSION

The palinological analysis of faeces samples of the *Megachile rotunadata* collected from colonies in the years 2003 and 2004 enabled identification of 46 plant species belonging to 23 families. In the years 1961-1977 Ruszkowski et al. (1980) stated that *Megachile rotunadata* visited 40 plant species from 13 families, mostly belonging to Fabaceae. Results of pollen analysis of faeces indicated that most of the visited taxa belonged to the Asteraceae family. Solitary bees preferred to visit *Melitotus*, *Trifolium repens*, *T. pratense*, *Vicia*, *Phaseolus* and *Medicago* from the Fabaceae family.

The pollen analysis of faeces of insects belonging to the Megachilidae family has not been used in identification of their food plants. Similar research, concerning identification of food plants visited by insects based on pollen analysis of their faeces, are known from only a few publications. Brian (1951) conducted palinological analysis of bumble bee larvae faeces. However, in the study, interference into the colony was necessary. Research by Kozłowska and Warakomska (1984) and Golding and Edmunds (2003) are concerned with plants visited by Diptera from the Syrphidae family. Anasiewicz (1992) investigated the contents of the digestive system of *Coccinella septempunctata*. The most similar methods, to those used in this research, are described

by Golding and Edmunds (2003). They obtained samples of faeces for analysis, without killing Diptera insects. These authors considered the life-saving character of the method.

The presented food supply sequence of *Megachile rotundata* based on the pollen analysis of their faeces has no equivalent in literature data.

Another very important aspect, is the fact that no single nest cell was damaged during the experiment. A negligible number (estimated for ca. 10%) of types of pollen grains that could not be identified in the study do not affect the practical applicability of the method.

CONCLUSIONS

1. The palinological analysis of faeces of *Megachile rotundata* enabled the identification of 46 taxa of visited plants.

2. The pollen analysis of faeces of solitary bees used for their food plants identification can be considered as life-saving as it does not interfere with the colony and consequently does not reduce the number of those advantageous insects.

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ROŚLINY POKARMOWE MIESIARKI LUCERNÓWKI (*Megachile rotundata* L.) OKREŚLONE NA PODSTAWIE ANALIZY PALINOLOGICZNEJ ODCHODÓW

Teper D.

S t r e s z c z e n i e

Próbki odchodów do badań pobierano w latach 2003-2004. W tym celu codziennie po zmroku przez cały okres lotu miesiarki lucernówki (*Megachile rotundata*) kolonie osłaniano, od strony wylotów z rurek, izolatorem z gęstej siatki. Następnego dnia rano pszczoły chcąc się wydostać na zewnątrz obsiadały izolator i wydalały odchody na jego powierzchnię.
Z odchodów wykonywano preparaty mikroskopowe, które poddano analizie palinologicznej.

Łącznie w latach badań w 56 preparatach mikroskopowych zidentyfikowano pyłek roślin należących do 23 rodzin. W zebranym materiale badawczym określono 46 różnych typów pyłku: typ *Achillea*, typ *Anthriscus*, Asteraceae, Brassicaceae, Brassicaceae małe, Brassicace duże, Caryophyllaceae, *Centaurea cyanus*, *Centaurea jacea*, *Chenopodium*, typ *Cichorium*, typ *Cirsium*, *Convolvulus*, *Crocosmia*, *Echium*, *Euphorbia*, Fabaceae, *Fuchsia*, Gramineae, typ *Helianthus*, typ *Heracleum*, *Impatiens*, Lamiaceae, *Lonicera*, *Lythrum*, typ *Malus*, *Medicago*, *Melilotus*, *Phacelia*, *Phaseolus*, *Phlox*, *Pinus*, Polygonaceae, Rosaceae, *Salvia*, typ *Solidago*, *Symphytum*, typ *Taraxacum*, *Teucrium*, *Tilia*, *Trifolium pratense*, *Trifolium repens* s.l., *Ulex*, *Vicia*, *Viola tricolor*, *Zea mays*. W odchodach pobranych do analiz w 2003 roku oznaczono 39 typów pyłku (Tab. 1), a rok później tylko 20 (Tab. 2). Pewnej, niewielkiej części pyłku nie można było oznaczyć ze względu na znaczne zniszczenie eksyny w procesie trawienia. Uszkodzone ziarna należały prawdopodobnie do gatunków roślin wytwarzających pyłek o cienkiej eksynie, najbardziej podatny na działanie warunków panujących w przewodzie pokarmowym owadów. Na podstawie szczegółowych analiz próbek odchodów murarki sporządzono dla każdego roku badań taśmę pokarmową, obrazującą zmiany w składzie gatunkowym oblatywanych roślin w ciągu sezonu (Tabela 1 i 2).

Słowa kluczowe: miesiarka lucernówka, *Megachile rotundata*, rośliny pokarmowe, analiza pyłkowa odchodów.