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ORIGINAL RESEARCH PAPER

Pollen sources in the Bojanów forest complex identified on honeybee pollen load by microscopic analysis

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Abstract

The aim of this study was to determine sources of pollen for the honeybee in the Bojanów forest complex, Nowa Dęba Forest District (southeastern Poland). Sampling of pollen loads from bees extended from the beginning of May until the end of September 2016 and was carried out at 7-day intervals using pollen traps mounted at the entrance of beehives. A total of 73 pollen load samples were collected from the study area.

Fifty-nine taxa from 31 plant families were identified in the analyzed material. From 4 to 21 taxa (average 9.5) were recorded in one sample. The pollen of Brassicaceae ("others"), *Taraxacum* type, *Solidago* type, and *Rumex* had the highest frequency in the pollen loads examined. Apart from these four taxa, pollen grains of *Rubus* type, Poaceae ("others"), *Calluna*, *Fagopyrum*, *Trifolium repens* s. l., *Phacelia*, *Aster* type, *Melampyrum*, *Quercus*, *Cornus*, and *Veronica* were recorded in the dominant pollen group. The forest habitat taxa that provided pollen rewards to honeybees in the Bojanów forest complex were the following: *Rubus*, *Calluna*, *Prunus*, *Tilia*, *Frangula alnus*, *Pinus*, *Quercus*, *Cornus*, *Robinia pseudoacacia*, *Salix*, and *Vaccinium*. Apart from forest vegetation, the species from meadows and wastelands adjacent to this forest complex, represented by *Taraxacum*, *Rumex*, *Plantago*, Poaceae, *Trifolium repens*, and *Solidago*, proved to be an important source of pollen. The study indicates that forest communities are a valuable source of pollen for pollinating insects from early spring through to late fall.

Keywords

Bojanów forest; bee food plants; pollen loads; pollen analysis; *Apis mellifera*

Introduction

Pollen is necessary for the nutrition of bees, for which it is the main source of protein, fats, vitamins, mineral salts, and also water [1]. A bee's life-span and its physiological state as well as the proper development of the pharyngeal glands of honeybee workers all depend on its consumption [2–4]. Pollen is carried to a hive in the form of loads and stored in the honeycomb cells. The number of larvae in a bee colony determines its collection and consumption [5]. The greatest demand for pollen occurs during the spring period, when bees need food to start a new season. According to Maurizio [3], pollen is characterized by its varying nutritional value. As far as this trait is concerned, this author distinguished three pollen groups, as of high, medium, or low nutritional value for bees. Among the taxa cited by Maurizio [3], there are pollen grains of forest trees and shrubs.

The aim of this study was to determine the flora supplying pollen for the honeybee in the Bojanów forest complex. It was based on microscopic analysis of the pollen content in pollen loads sampled at different periods of the flowering season.

Material and methods

This study was conducted in the Bojanów forest complex, Nowa Dęba Forest District (southeastern Poland). Within the area, natural forest complexes and man-made forests are found. Fresh pine forests (*Leucobryo-Pinetum*) is the most frequent type of vegetation. In particular, a number of shrub layer species and ground layer herbs are recognized as attractive food plants for the honeybee, *Apis mellifera*.

Bee colonies were established at four forest sites, taking into account the average flight range of the honeybee so as to ensure that the largest possible area of the forest was within their foraging range. Two hives with bees were placed at each of the four selected sites (Fig. 1). Pollen loads were sampled at 7-day intervals, from the beginning of May to the end of September 2016, using pollen traps mounted at the entrance of the beehives. Samples collected in May and June were considered to be spring samples, those collected in July and August were summer samples, whereas those collected in September were considered to be examples of fall pollen rewards. Each sample was carefully mixed, weighed, and subsequently 3.5-g portions were used when preparing pollen loads for microscopic analysis. The weighed portions of pollen loads were dispersed in 10 mL of 1:1 mixture of distilled water and glycerol. Next, microscope slides were prepared by the Smaragdova method [6]. Microscopic analysis was performed using a Nikon Eclipse E 600 light microscope at a magnification of 40×15. According to the recommendations of Moar [7], at least 300 pollen grains were counted in two replicates for each slide. An effort was made to assign pollen to the most accurate taxon (species, genus, structure type, or family) using Zander's classification [8]. In order to identify a pollen taxon as accurately as possible, keys for identification of pollen grains were developed [8–10]. Unidentified pollen grains within a family were classified as "others"; for example, among Brassicaceae the following were identified: *Brassica napus*, *Sinapis alba*, and Brassicaceae ("others"), among Poaceae: *Zea mays* and Poaceae ("others").

Following identification of the pollen grains, percentages of the individual taxa were calculated, classifying them into four groups: dominant pollen with a percentage >45% in the sample, accessory pollen with a percentage from 16% to 45%, isolated pollen from 3% to 16%, and occasional pollen whose percentage in the sample was <3%. On the basis of the pollen spectrum of each sample, the frequency and percentage of the individual taxa were calculated for the whole material as well as for the samples collected in spring, summer, and fall. Photographic documentation of the microscopic images of analyzed samples of bee pollen loads was made using Lucia Screen Measurement image analysis software.

Results

A total of 73 pollen load samples were obtained from the Bojanów forest complex, including nine in May, 12 in June, 20 in July, and 16 samples in both August and September. The weight of individual samples ranged from 3.65 to 107.80 g (average 26.21 g). Pollen loads collected in spring had the greatest weight, ranging from 3.92 g to 107.80 g (average 41.55 g). In summer, the weight was lower, from 3.65 g to 95.50 g (average 22.30 g). For all the samples taken, the lowest weight was recorded in September, when individual samples weighed from 4.30 g to 38.40 g (average 17.77 g).

Pollen grains of 59 taxa were distinguished in the material analyzed, including 44 originating from nectar-producing plants and 15 from nectarless plants (anemophilous and entomophilous taxa) (Fig. 2). Pollen loads collected in July and August had the highest number of taxa identified. During this period, pollen belonging to 44 taxa (33 nectar-producing plants and 11 nectarless) was distinguished in the pollen loads examined. A lower number of taxa was recorded in pollen loads sampled in May and June – pollen grains of 36 taxa, including 26 nectar-producing and 10 nectarless plants, were identified. The lowest number of pollen taxa was found in pollen loads captured in September; 22 pollen taxa were distinguished, among them 17 from nectar-producing plants and five from nectarless ones. The number of pollen taxa recorded per sample ranged between 4 and 21, 9.5 on average. The highest average number of identified taxa was in pollen load samples collected in summer (11.2), whilst the lowest one was

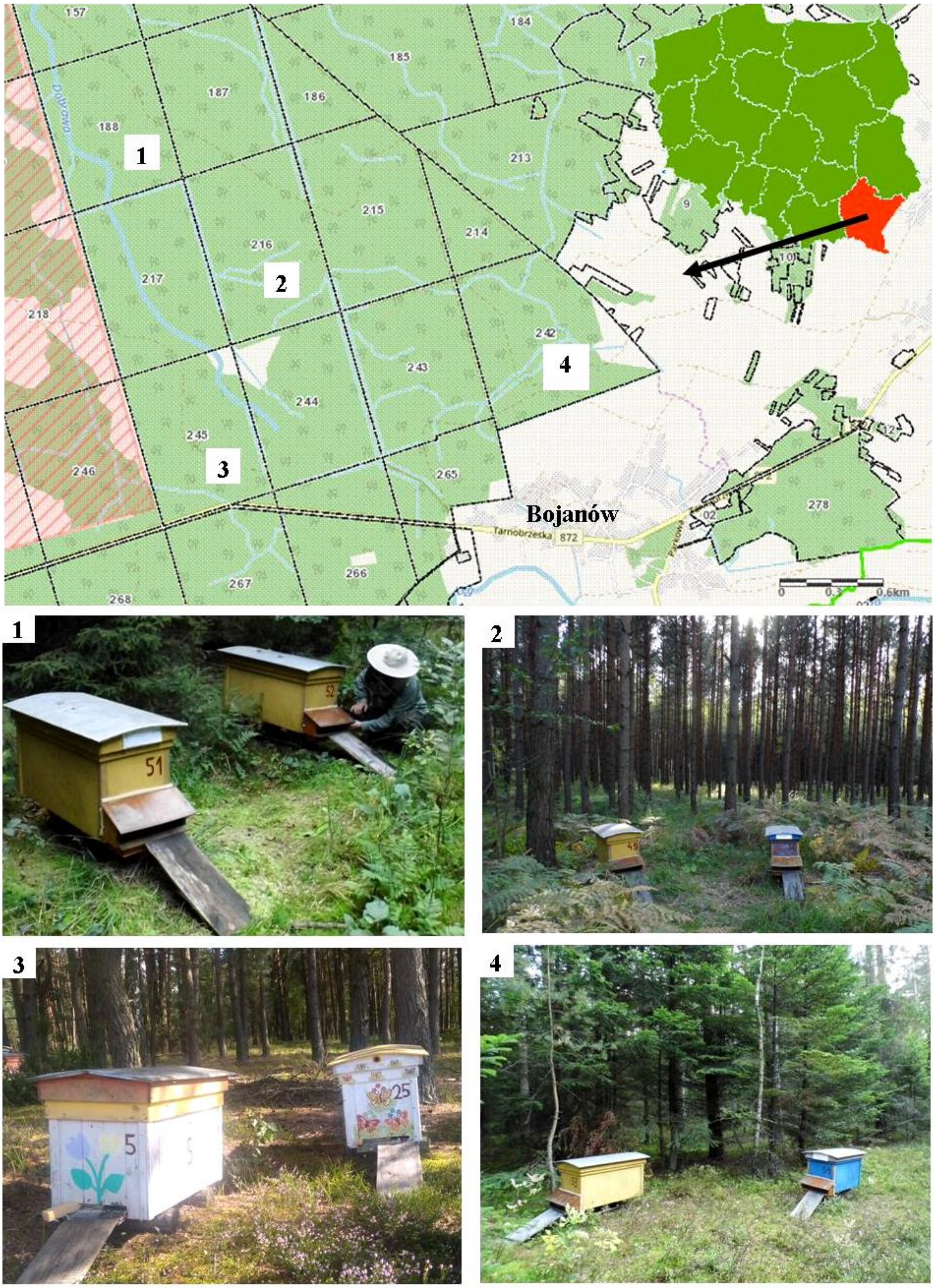

Fig. 1 Map and fragments of study area with beehives.

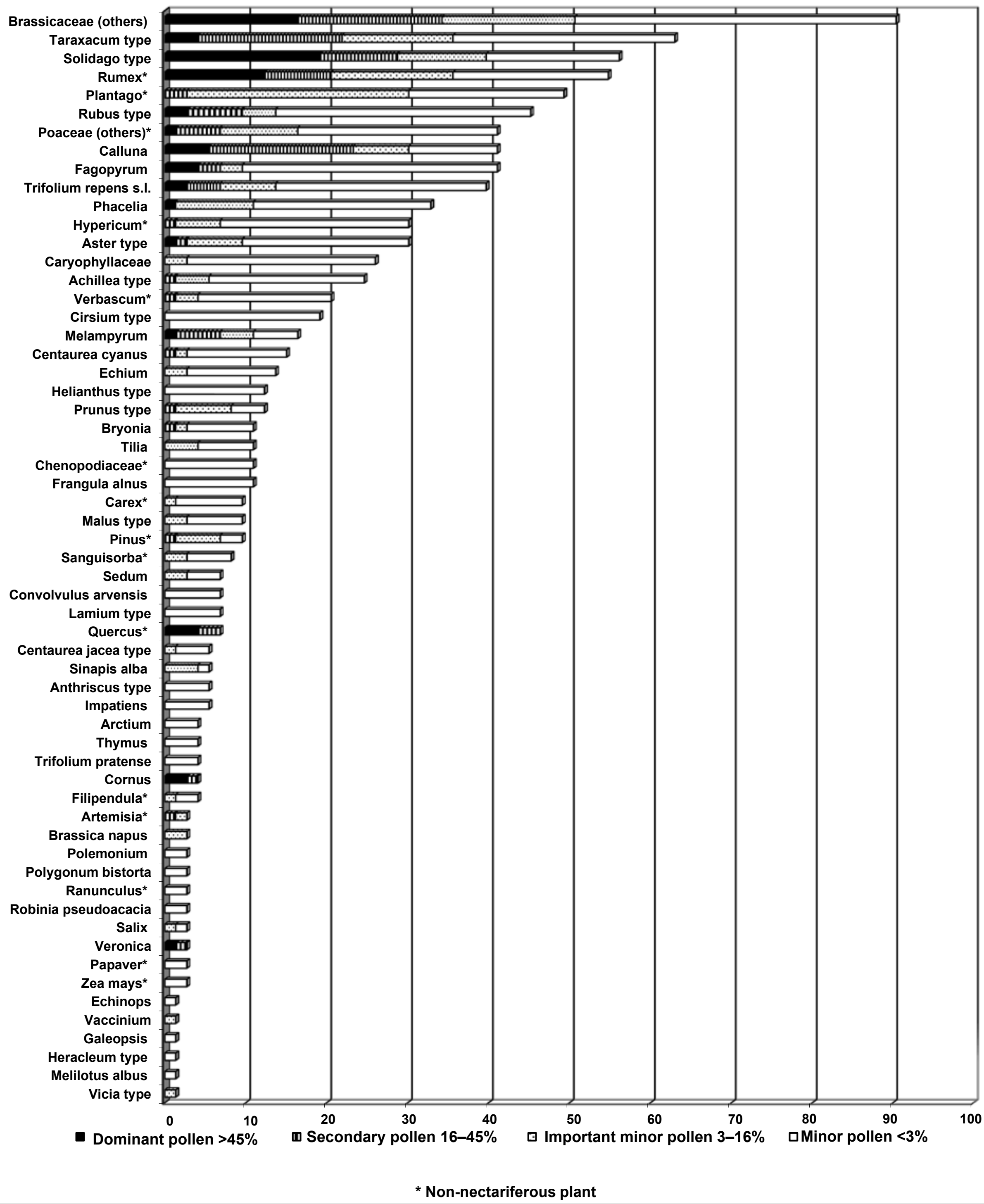

Fig. 2 Pollen frequency (%) of 73 pollen loads samples and its participation (%) in microscopic view.

in those taken in fall (6.4). In spring, the average number of taxa identified per sample was 9.0. The pollen taxa identified originated from 31 plant families. The Asteraceae, from which 11 pollen taxa were found, as well as the Fabaceae and Rosaceae with five taxa from each of these families, were represented in greatest numbers. Three taxa were recorded from the families Brassicaceae, Lamiaceae, and Polygonaceae, and two taxa from the Apiaceae, Ericaceae, Poaceae, and Scrophulariaceae. Only one pollen taxon was identified for each of the other 21 families.

The pollen of Brassicaceae ("others") – 90.4%, *Taraxacum* type – 63.0%, *Solidago* type – 56.2%, and *Rumex* – 54.8%, had the highest frequency in the samples examined (Fig. 2). The pollen of Brassicaceae ("others") also had a high frequency in each period of pollen load sampling. In spring, it was 90.5%, in summer 72.2%, whereas in fall it was 75.0% (Tab. 1). In May and June, apart from the pollen of Brassicaceae ("others"), *Rubus* type – 80.9%, *Rumex* – 71.4%, and Poaceae – 66.7% also showed a high frequency, whilst in summer *Taraxacum* type – 72.2%, *Solidago* type – 69.4%, *Plantago* and *Rumex* – 68.9% each, *Fagopyrum* – 58.3%, *Calluna* – 55.6%, *Hypericum* – 52.8%, and *Trifolium repens* s. l. – 50.0% were recorded (Tab. 1). In September, *Solidago* type, which was found in each pollen load sample, exhibited a higher frequency compared to the summer frequency. A similar trend was noted in the case of *Taraxacum*-type pollen, whose frequency in fall was 87.5%. In this period, the pollen of *Aster* type and *Calluna* was also found frequently in pollen loads. The frequency of these taxa in pollen loads sampled in September was 62.5% for both of them (Tab. 1).

In the dominant pollen group (a percentage >45% in the sample), the following taxa were recorded: Brassicaceae ("others"), *Taraxacum* type, *Solidago* type, *Rumex*, *Rubus* type, Poaceae ("others"), *Calluna*, *Fagopyrum*, *Trifolium repens* s. l., *Phacelia*, *Aster* type, *Melampyrum*, *Quercus*, *Cornus*, and *Veronica* (Tab. 1, Fig. 2). In spring, the pollen of Brassicaeae ("others"), *Rubus* type, Poaceae ("others"), *Trifolium repens* s. l., *Phacelia*, *Fagopyrum*, *Quercus*, and *Cornus* had a dominant percentage (Tab. 1). In summer, *Taraxacum* type, *Solidago* type, *Rumex*, *Fagopyrum*, *Calluna*, *Melampyrum*, and *Aster* type were found in the dominant pollen group (Tab. 1). In fall, apart from *Solidago* type and *Aster* type, the dominant percentage of *Veronica*, in spite of its low frequency (12.5%), is notable (Tab. 1). Fig. 3 shows a fragment of the microscopic image of the pollen spectrum and of the most important bee taxa occurring in the Bojanów forest complex.

The following plant taxa provided pollen rewards for honeybees in the Bojanów forest complex: *Rubus*, *Calluna*, *Prunus*, *Tilia*, *Frangula alnus*, *Pinus*, *Quercus*, *Cornus*, *Robinia pseudoacacia*, *Salix*, *Vaccinium*, *Melampyrum*, *Anthriscus*, *Impatiens*, *Galeopsis*, and *Heracleum*. During the spring period, the following were of importance: *Rubus*, *Prunus*, *Pinus*, *Tilia*, *Quercus*, *Frangula alnus*, *Cornus*, *Robinia pseudoacacia*, *Salix*, *Vaccinium*, and also *Anthriscus* and *Lamium*. The following taxa were considered to be the most important in July and August: *Rubus*, *Calluna*, and *Melampyrum*, while *Calluna* in fall.

In the study area, the following plants from the meadows and wastelands adjacent to the forest were an important source of pollen: *Taraxacum*, *Rumex*, *Plantago*, Poaceae, *Trifolium repens* s. l., Caryophyllaceae, *Achillea*, *Cirsium*, *Carex*, *Sanguisorba*, *Sedum*, *Convolvulus arvensis*, *Lamium*, *Centaurea jacea*, *Trifolium pratense*, *Filipendula*, *Polygonum bistorta*, *Ranunculus*, *Veronica*, *Papaver*, *Melilotus*, *Vicia*, and *Veronica*. Bees used these pollen sources especially readily in summer and fall. The pollen resources were complemented by crop plants, weeds, herbs as well as orchard and garden plants occurring in the vicinity.

Discussion

The microscopic analysis of the pollen loads showed that during the early spring period the trees *Quercus*, *Cornus*, *Pinus*, and *Salix* were the main source of pollen in the Bojanów forest complex. According to Maurizio [4], honeybees use the pollen of *Quercus* and *Pinus* despite it having a low nutritional value. In Poland, the interest of bees in collecting oak pollen was confirmed by Wróblewska et al. [11], who found *Quercus* pollen grains in bee bread from the northeastern region of Poland. Sá-Otero et al. [12] also demonstrate that *Quercus* pollen occurred in pollen loads of honeybees in northwestern Spain, whereas Šaulienė et al. [13] found its presence in pollen loads in Lithuania. Maurizio and Grafl [14] stress that honeybees do not readily visit male inflorescences of *Pinus*. *Cornus* pollen grains were found by Wróblewska [15] in the microscopic image of bee bread from the Lublin region.

Our study revealed that *Rubus* shrubs and *Prunus* trees were the main spring source of pollen in the Bojanów forest complex. A study by Szklanowska and Wieniarska [16] confirmed the high attractiveness of raspberries and blackberries as a source of pollen for bees in SE Poland. The pollen of *Rubus* type in pollen loads of bumblebees in Poland was identified by Teper [17]. Wróblewska [15] and Wróblewska et al. [11] found raspberry and blackberry pollen in bee bread from the area of Podlasie and northeastern Poland.

Tab. 1 Major polleniferous plants in the examined area on the basis of pollen frequency and participation in pollen load samples.

Taxon	Frequency (%)	Pollen participation per sample: dominant >45%	Pollen participation per sample: secondary 16–45%
May–June			
Brassicaceae ("others")	90.47	28.57	9.52
Rubus type	80.95	9.52	23.81
*Rumex**	71.43	-	-
Poaceae ("others")*	66.66	4.76	9.52
Phacelia	33.33	4.76	-
Fagopyrum	23.81	4.76	4.76
*Quercus**	23.81	14.29	9.52
Cornus	14.29	9.52	4.76
Prunus type	12.33	-	1.37
July–August			
Brassicaceae ("others")	72.23	-	-
Taraxacum type	72.23	2.78	5.56
Solidago type	69.44	30.55	5.56
*Plantago**	63.89	-	5.56
*Rumex**	63.89	25.00	16.67
Fagopyrum	58.34	5.56	2.78
Calluna	55.56	2.78	22.22
*Hypericum**	52.78	-	2.78
Trifolium repens s. l.	50.00	-	8.33
Melampyrum	33.33	2.78	11.11
Aster type	30.56	2.78	2.78
September			
Solidago type	100.00	18.75	31.25
Taraxacum type	87.50	12.50	6.25
Brassicaceae ("others")	75.00	37.50	12.50
Aster type	62.50	-	-
Calluna	62.50	18.75	31.25
Veronica	12.50	6.25	-

* Non-nectariferous plant.

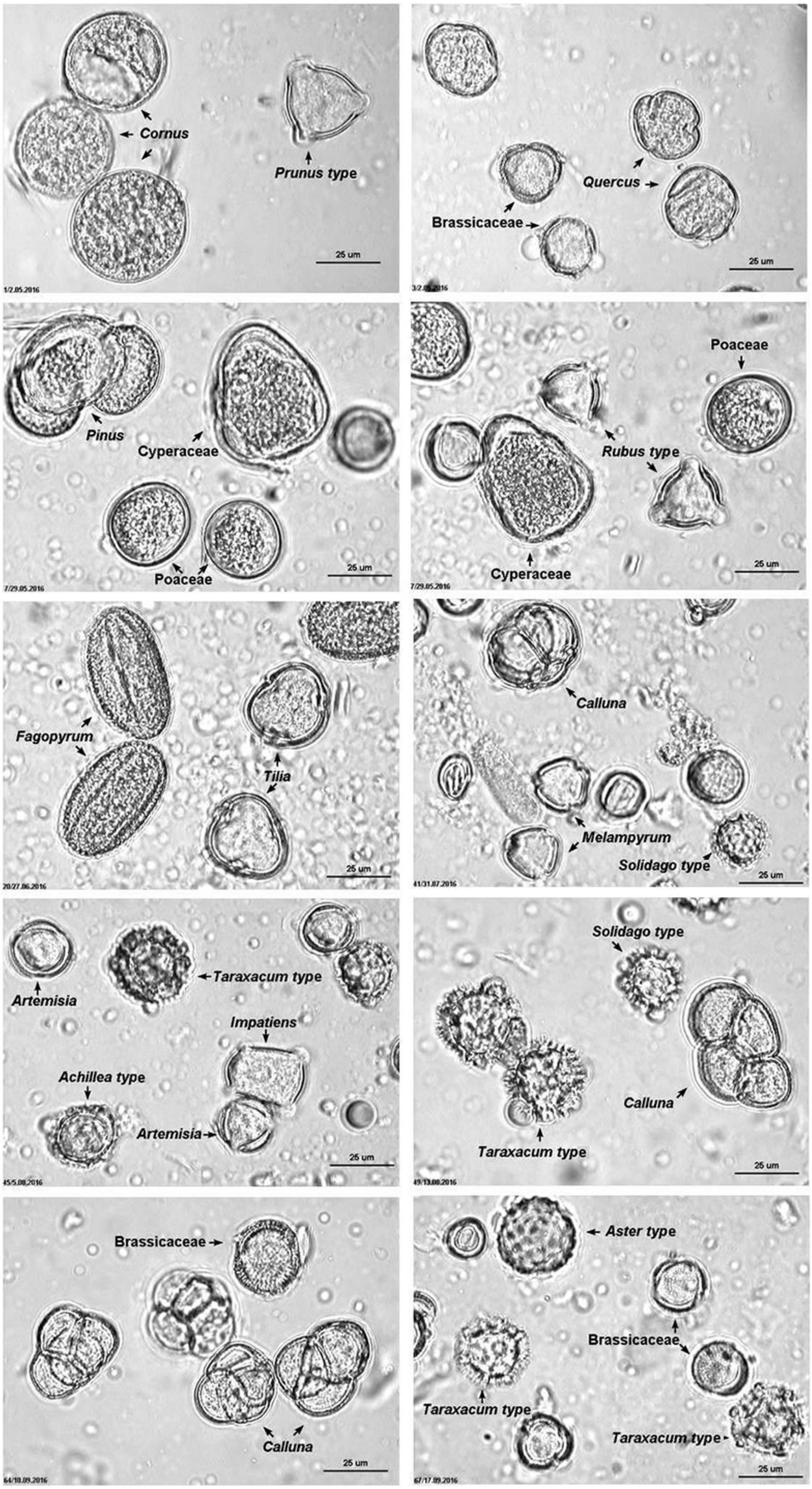

Fig. 3 A part of microscopic view of some pollen loads sample.

Ortiz and Polo [18] and Sá-Otero et al. [12] recorded *Rubus* pollen grains in pollen loads of the honeybee in Spain as did Beil et al. [19] in Germany.

Pollen of *Prunus* type was recorded by Wróblewska [15] in bee bread from the Lublin region, and Klepacz-Baniak and Czekońska [20] found it in pollen loads sampled in the area of Cracow. Moreover, pollen of this taxon was found by Beil et al. [19] in pollen loads in Germany. *Prunus*-type pollen also occurred in pollen loads from the period of April and May sampled in the northeastern area of Spain [12] and has also been recorded from Islamabad, Pakistan [21].

Apart from trees, within the study area valuable pollen rewards were provided by herbaceous plants growing in the meadows adjacent to the forest, among them various species of the Brassicaceae and Poaceae, *Rumex*, *Plantago*, *Trifolium repens*, and *Taraxacum*. The high frequency and participation of Brassicaceae pollen was recorded in pollen samples collected in Bojanów during each part of the flowering season. The attractiveness of this pollen source for honeybees was also described by Warakomska et al. [22] in the microscopic image of bee bread from the Moravian Valley area. Similarly, Wróblewska [15] and Wróblewska et al. [11] found that the Brassicaceae is one of major plant families that provides pollen rewards in the area of Podlasie and northeastern Poland. Denisow [23,24] also emphasized the great importance of plants of the Brassicaceae as a source of pollen. According to Noor et al. [21], plants of this family are also the main source of pollen in Islamabad (Pakistan). Atanassova and Lazarova [25] further stress their great apicultural importance in Bulgaria.

The importance of Poaceae, *Rumex*, and *Plantago* as a source of pollen for insects is confirmed by a study by Maurizio and Grafl [14], according to whom bees show interest in the pollen of these anemophilous taxa, especially as they bloom for a long time and in abundance. The research of these authors showed that in Switzerland, grass pollen accounted for about 15% of pollen rewards collected by bees during a season. A high frequency and a dominant percentage of Poaceae pollen in the microscopic slides of bee bread from the Moravian Valley was also found by Warakomska et al. [22], whilst Wróblewska [15] and Wróblewska et al. [11] observed the same for bee bread from the area of Podlasie and northeastern Poland. This is also confirmed by the reports of Sá-Otero et al. [12] from northwestern Spain, by Atanassova and Lazarova [25] from Bulgaria, and by Šaulienė et al. [13] from Lithuania. These authors very frequently recorded Poaceae pollen in the microscopic image of pollen loads collected from bees.

In the Bojanów forest complex, large amounts of pollen are provided by *Rumex* and *Plantago*. A distinct presence of pollen grains of these plants can frequently be seen in bee bread and pollen loads from Poland [11,15,17,26,27], Switzerland [14], and Bulgaria [25].

Trifolium repens deserves attention among nectar-producing plants from the study area adjacent to the forest. In the analyzed material, the pollen of *T. repens* reached nearly 40% frequency and its percentage contribution classified it as the dominant or accessory pollen group. Maurizio [4] includes clover pollen in the group with the highest nutritional value for bees. Demianowicz and Warakomska [28] recorded a high frequency of *T. repens* s. l. pollen in bee bread from the Vistula Spit, and Wróblewska [15] and Wróblewska et al. [11] in bee bread and pollen loads from the Podlasie region and northeastern Poland. Klepacz-Baniak and Czekońska [20] observed the presence of *Trifolium* pollen grains in pollen loads from the area of Cracow. Pelimon [29] found that in Romania, *Trifolium* was the main source of pollen for bees. Parent et al. [30] noted the pollen of *T. repens* s. l. in pollen loads in their study in Canada, and Noor et al. [21] in Islamabad (Pakistan).

In the study area, pollen grains of *Taraxacum* type reached a high frequency and percentage participation in pollen loads sampled in July, August, and September. The attractiveness of *Taraxacum*-type pollen as a source of food for insects was confirmed by research conducted in Poland [11,15,20] and in other European countries [19,25]. The *Taraxacum*-type pollen was noted at high frequency in the pollen loads. *Hypericum*, *Melampyrum*, and *Veronica* showed a lower frequency but a high percentage (in the dominant and accessory pollen groups) in the pollen loads analyzed. According to Szklanowska and Denisow [31], *Hypericum* can provide as much as 15 kg of pollen per 1 ha. Kołtowski and Jabłoński [32] demonstrated that St. John's wort is an important supplementary source of pollen for insects in wastelands. Wróblewska [11] found *Hypericum* pollen grains in both bee bread and pollen loads collected in northeastern

Poland. The interest of bees in *Melampyrum* and *Veronica* pollen was confirmed by Young and Young [33], who found the presence of pollen grains of the these taxa in the pollen loads.

Calluna and species of the Asteraceae growing in the Bojanów forest complex, including *Cirsium*, *Solidago*, and *Aster*, provided pollen at the end of summer and in fall. The attractiveness of pollen rewards from *Calluna* for the honeybee was reported by Serwatka [34], Poszwiński and Warakomska [35], Maurizio [36] as well as by Luis-Villota and Gomez-Ferreras [37]. These authors write only about varietal heather honeys, but they did not study pollen loads or bee bread. According to Maurizio [4], *Calluna* pollen is amongst the pollen taxa with the highest nutritional value for bees. Diaz-Losada et al. [38] found heather pollen grains in pollen loads from Spain and Italy. Furthermore, Diaz-Losada et al. [38] and Sá-Otero et al. [12] both recorded *Erica cinerea* and *E. umbellata* pollen grains in pollen loads from the northwestern area of Spain.

The image of pollen loads from Bojanów showed a high presence of pollen from the Asteraceae supplying food mainly in summer and fall. Young and Young [33], Muszyńska and Warakomska [39,40], Beil et al. [19], Atanassova and Lazarova [25], and Šauliené et al. [13] all found pollen grains from the Asteraceae in both pollen loads and bee bread. Based on an analysis of bee bread, Demianowicz and Warakomska [28] included goldenrod in the main pollen-providing plants in the Vistula Spit area, and its great apicultural importance was confirmed by a study by Strzałkowska [41].

Fagopyrum and *Phacelia* are typical agricultural plants growing within the Bojanów forest complex area. These species also provided pollen rewards for honeybees. Pollen grains of *Fagopyrum* and *Phacelia* were recorded by Warakomska and Muszyńska [42] in pollen loads from the area of Puławy, by Klepacz-Baniak and Czekońska [20] in pollen loads from the Cracow area, and by Wróblewska et al. [11] in bee bread from northeastern Poland. *Fagopyrum* pollen grains also reached a high frequency in the microscopic image of bee bread from the Podlasie area [15]. The study by Björkman [43] also confirms the attractiveness of this taxon for insects.

Conclusion

The flora occurring in the studied area provides a diversified source of pollen flow for pollinating entomofauna. Pollen taxa presented in pollen loads came from plants blooming from early spring to late fall, supplying insects with pollen food throughout the growing season.

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Źródła pożytku pyłkowego kompleksu leśnego Bojanów na podstawie analizy mikroskopowej obnóży pyłkowych pszczoły miodnej

Streszczenie

Celem pracy było określenie źródła pożytku pyłkowego pszczoły miodnej w kompleksie leśnym Bojanów, nadleśnictwo Nowa Dęba (południowo-wschodnia Polska). Pobieranie prób obnóży pyłkowych od pszczół trwało od początku maja do końca września 2016 roku. Materiał pobierano, w siedmiodniowych odstępach czasu, stosując na ulach wylotowy poławiacz pyłku. Ogółem z terenu badań pozyskano 73 próbki obnóży pyłkowych.

Analizę mikroskopową obnóży wykonano przy użyciu mikroskopu świetlnego Nikon Eclipse E600 przy powiększeniu 40×15. W każdym preparacie liczono co najmniej 300 ziaren pyłku w dwóch powtórzeniach po 150 ziaren w każdym z nich. Na podstawie spektrum pyłkowego każdej próbki obliczano frekwencję i udział poszczególnych taksonów w całości materiału, a także w próbkach pozyskanych wiosną, latem i jesienią.

W analizowanym materiale wyróżniono ziarna pyłku 59 taksonów z 31 rodzin botanicznych. W jednej próbce notowano od 4 do 21 taksonów (średnio 9.5). W badanych obnóżach pyłkowych najwyższą frekwencję osiągnął pyłek Brassicaceae (inne), typu *Taraxacum*, typu *Solidago* i *Rumex*. W grupie pyłku dominującego (udział powyżej 45% w próbce) poza wspomnianymi 4 taksonami notowano jeszcze pyłek typu *Rubus*, Poaceae (inne), *Calluna*, *Fagopyrum*, *Trifolium repens* s. l., *Phacelia*, typu *Aster*, *Melampyrum*, *Quercus*, *Cornus* i *Veronica*.

Gatunkami siedlisk leśnych dostarczającymi pszczole miodnej pożytku pyłkowego w kompleksie leśnym Bojanów były: *Rubus*, *Calluna*, *Prunus*, *Tilia*, *Frangula alnus*, *Pinus*, *Quercus*, *Cornus*, *Robinia pseudoacacia*, *Salix* i *Vaccinium*. Poza roślinnością leśną istotnym źródłem pyłku okazały się gatunki graniczących z kompleksem leśnym łąk i nieużytków reprezentowane przez: *Taraxacum*, *Rumex*, *Plantago*, Poaceae, *Trifolium repens* i *Solidago*.

Przeprowadzone badania dowodzą, że zbiorowiska leśne stanowią cenne źródło pożytku pyłkowego dla owadów zapylających. Pożytek ten jest dostępny od wczesnej wiosny do późnej jesieni.