

## FOOD PLANTS OF *Bombus terrestris* L. AS DETERMINED BY POLLEN ANALYSIS OF FAECES

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### S u m m a r y

In 2002 – 2004, faeces samples were collected to paper pockets stucked to the wall under the entrance to wooden hives settled by colonies of *Bombus terrestris*. A total of 105 faeces samples were collected during three years study and microscope preparations were made thereof. In the samples 56 pollen grains types belonging to 28 families were identified. The majority of the identified pollen types came from entomophilous taxons. A certain part of pollen could not be identified because of its extensive destruction during digestion. For each study year, based on pollen analysis data a food supply sequence for *Bombus terrestris* was worked out.

**Keywords:** *Bombus terrestris*, food plants, pollen analysis of faeces, digestion of pollen.

### INTRODUCTION

Bumblebees (*Bombus* Latr.) are the second most important pollinators next to the honeybee. Their particular significance in the pollination of especially forage legumes such as *Trifolium*, *Vicia*, *Medicago*, *Lotus* have been confirmed in numerous studies (i.a. Ruszkowski and Biliński 1969, Ruszkowski 1971). Analysis of the pollen and faeces of the larvae collected from bumblebee nests conducted by Brian (1951), as well as pollen analysis of pollen loads of the bumblebees captured on flowers of diverse plant species (Anasiewicz and Warakomska 1977, Warakomska and Anasiewicz 1991) substantially extended the list of known forage plants for those species. The interest in bumblebees received a further boost when they were introduced to pollinate greenhouse-grown crops, especially tomatoes. *Bombus terrestris* turned out to be of particular value as it is relatively easy to rear.

Ruszkowski (1998) drew up an all-season list of indicator species for short-tongued bumblebees *Bombus terrestris* and *B. lucorum* based on his own

observations and literature data. In a collective food supply sequence for *Bombus terrestris* and *B. lucorum* (Ruszkowski and Żak 1974) which was drawn up based on observations conducted in the years 1961 – 1970 the investigators named 52 taxons visited by those species in the province of Lublin.

In a study on the visitation of *Medicago media* by bumblebees based on the pollen analysis of pollen loads made by bumblebees, Anasiewicz and Warakomska (1969) supplied evidence that lucerne provided the major source of forage for *Bombus terrestris*, *B. lucorum* and *B. hypnorum*. In another study of those investigators (1976) bumblebees were found to be the most numerous among wild apiform insects which pollinated *Trifolium pratense* as they accounted for 83% of total captured individuals. Instead, pollen analysis of the pollen collected showed that red clover was the most frequently visited by *Bombus hortorum*, *B. equestris* and *B. muscorum*. The study also proved that pollen analysis is a more effective tool to identify forage plants for insects as com-

pared to observational methods since an insect recorded on a given plant had pollen from other species in its pollen loads. It was also confirmed by the pollen analysis of pollen loads made by bumblebees captured on *Vicia villosa* i *Vicia sativa* (Warakomska and Anasiewicz 1991).

In a study aimed at identifying forage plants of bumblebees (*Bombus* Latr.) in the province of Lublin (Anasiewicz, Warakomska 1977) a total of near 1,500 insects were captured. Of those 189 were pollen foragers of which 48 belonged to *B. terrestris*. In pollen loads of this species analyzed for pollen type 37 types from 18 plant families were found. *Trifolium pratense* was the main source of pollen. The pollen of *Lotus corniculatus*, *Trifolium repens*, *Hypericum*, *Papaver* and *Sinapis arvensis* was also frequent.

Based on the pollen analysis of pollen loads collected from nest-bound worker bumblebees *Bombus terrestris* the author found 29 plant species as being visited by the insects (Teper 2004). They included 9 taxons the bumblebees of which made homogeneous pollen loads and those species were recognized as the main forage plants of *B. terrestris*. They were Brassicaceae, *Centaurea cyanus*, *Echium vulgare*, *Filipendula ulmaria*, *Lotus corniculatus*, *Hypericum*, *Plantago*, *Trifolium pratense*, *T. repens*. In that study the author also drew the attention to a greater efficiency of pollen analysis and to a lower labour requirement of the method as compared to observational based methods.

The aim of the study was to verify if the pollen analysis of faeces, which is safe for the insects and less labour-consuming than observational methods may be useful in the identification of plants visited by *Bombus terrestris*.

## MATERIAL AND METHODS

In the research colonies of *Bombus terrestris* originating from a culture run for many years at Puławy were used. The species is used on a large scale in pollination of greenhouse-grown crops.

Before the regular study was started several microscope preparations of bumblebee faeces sampled from the reared colonies were prepared. Their analysis showed that the majority of pollen grains look almost the same as the grains from reference preparations.

In the study conducted in the years 2002 – 2004 bumblebee colonies were placed in Górna Niwa Exp. Sta. and in an area adjacent to Marynki Palace - the quarters of the Apiculture Division in Puławy.

The observation of a dozen or so *Bombus terrestris* colonies originating from cultures placed in mating isolators yielded evidence that all styrofoam colony-containing hives are, to a larger or smaller extent, contaminated with faeces on their front wall under the entrance. Because of that the spot was thought to be the best for sampling of faeces. To this end, special “pockets” were made of self-sticking paper tape which were stuck right under the entrance to the wooden hive settled by a bumblebee colony. The “pockets” were changed daily starting from the day on which the colonies were brought out: July 13, 2002, May 19, 2003 and June 16, 2004 and until they died down 30 – 56 days later. The bumblebee faeces accumulated in the “pocket”, mostly in semi-liquid state, were transferred to labelled small bottles and placed in a refrigerator. Because of the very rich microflora of the faeces their moulding proceeds very fast hence microscope preparations ought to be made on the very same day. Due to organizational considerations it was not always possible and therefore to each bottle a small crystal of phenol (C<sub>6</sub>H<sub>5</sub>OH), a potent microbial in-

hibitor, was added and larger batches of microscope preparations were made on a later date.

Prior to making microscope preparations an appropriate amount of distilled water was added to each bottle to roughly equalize the dilution of each sample and the content of the bottle was mixed by energetic shaking. Diluted faeces were smeared three times with a preparation loop onto the surface of a microscopic slide that was labelled with detailed information on insect species, site, and sampling date. After being dried lightly, each preparation was added a drop of glycerol-gelatin and closed with a cover slip. Glycerol-gelatin was prepared according to the protocol described by Sawyer (1981). The identification of pollen grains in microscope preparations was done using available pollen atlases (i.a. Sawyer 1981, 1988; Ricciardelli d'Albore 1998; Bucher et al. 2004) and the author's own collection of more than 300 reference preparations using Zander's classification (1935, 1937, 1941, 1949, 1951).

In each microscopic slide pollen was identified if possible down to species, genus, family or structure type within a family.

## RESULTS

A total of 56 pollen grain types belonging to 28 families were identified in 105 faeces samples collected from the *Bombus terrestris* over three years of the study: *Achillea* type, *Anthriscus* type, Asteraceae, *Aesculus*, *Artemisia*, *Borago*, Brassicaceae, *Calystegia*, *Centaurea cyanus*, *C. jacea*, *C. scabiosa*, *Chenopodium*, *Cichorium* type, *Convolvulus*, *Cornus*, *Dipsacus*, *Dracocephalum*, *Echinops*, *Echium*, Ericaceae, *Fagopyrum*, *Filipendula*, *Frangula*, *Geranium*, *Helianthus* type, *Heracleum* type, *Impatiens*, *Knautia*, Lamiaceae, *Lonicera*, *Lotus*, *Lythrum*,

Malvaceae, *Medicago*, *Melilotus*, Onagraceae, *Phacelia*, *Pinus*, *Plantago*, *Polygonum*, *Reseda*, Rosaceae, *Rubus* type, *Rumex*, *Salvia* type, *Sambucus*, *Solidago* type, *Symphytum*, *Taraxacum* type, *Tilia*, *Trifolium pratense*, *Trifolium* type, *Viburnum*, *Vicia*, *Viola tricolor*, *Zea mays*. Thirty-four types were identified in bumblebee faeces in 2002 (Table 1), 29 types in 2003 (Table 2), and only 24 types in 2004 (Table 3).

The majority of the pollen types belonged to entomophilous species and some of anemophilous plants, for example: *Zea mays*; *Pinus*; *Plantago* and *Rumex*. A certain part of pollen samples could not be identified due to a large extent of damage to the grains during digestion. The damaged grains probably belonged to plants that formed pollen with very thin exine, such grains being most vulnerable to the conditions in the digestive tract of insects.

Based on the detailed analysis of bumblebee faeces samples a succession of forage plants was drawn up for each year of the study (Table 1, 2, 3) according to the method of Ruszkowski and Žak (1974) that presents the changes in the specific composition of visited plants over the season.

## DISCUSSION

The study resulted in identification of 56 pollen types that belonged to plants of 28 families. A slightly smaller number of taxons visited by bumblebee is reported by Ruszkowski and Žak (1974) in a food timeline for that species drawn up based on 9 years of observations and capture of specimens. The majority of bumblebee food plants as reported by those investigators *Aesculus*, *Dracocephalum*, *Echinops*, *Helianthus*, *Medicago*, *Salvia*, *Solidago*, *Tilia*, *Trifolium pratense*, *T. repens*, *Vicia* were also identified in this study based on pollen analysis of faeces. Such similarities







can be also found when comparing the results with the list of indicator plants for short-tongued bumblebees (Ruszkowski 1998) as well as with the results of Anasiewicz and Warakomska (1977), Warakomska and Anasiewicz (1991), Brian (1951) and the author's (Teper 2004). Noticeable differences in the specific makeup between the results of different studies may probably been caused by the floristic diversity of the study area and by different observation dates.

Another important aspect is the fact that the list of plants visited by the insects based on pollen analysis of the faeces was drawn up without capturing any individual whereas Warakomska and Anasiewicz (1991) in their two-year pollen analysis study of pollen loads made by bumblebees captured a total of 464 individuals. In another study aimed at identification of food plants for bumblebees in the province of Lublin Anasiewicz and Warakomska captured nearly 1,500 individuals. Nor was the life of bumblebees interfered with in the faeces-based study described in this paper unlike in that by Brian (1951) who collected the faeces for her study from insect nests.

A small number pollen grain types, estimated at 10%, that the author failed to identify had no influence on the efficacy and practical application of the method.

### CONCLUSIONS

1. Pollen analysis of *Bombus terrestris* faeces conducted over three seasons showed the presence of 56, mainly nectariferous, plant taxons visited by that species.
2. The majority of pollen grains observed in the faeces was little damaged and thus identifiable.
3. Less than 10% of pollen grains could not be identified due to extensive damage during digestion.
4. The method presented in the study is applicable in the researches of food plants for insects, including rare and protected species, since it does not involve capturing insects or nest destruction.

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## ROŚLINY POKARMOWE TRZMIELA ZIEMNEGO (*Bombus terrestris* L.) OKREŚLANE NA PODSTAWIE ANALIZY PALINOLOGICZNEJ ODCHODÓW

T e p e r D .

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

Próbki odchodów pobierano w latach 2002-2004 do papierowych torebek przyklejanych na ścianie pod wylotkiem drewnianych ulików zasiedlonych przez rodziny trzmiela ziemnego (*Bombus terrestris*). Łącznie w ciągu 3 lat badań w 105 próbkach odchodów oznaczono 56 typów ziaren pyłku należącego do 28 rodzin: typ *Achillea*, typ *Anthriscus*, Asteraceae, *Aesculus*, *Artemisia*, *Borago*, Brassicaceae, *Calystegia*, *Centaurea cyanus*, *C. jacea*, *C. scabiosa*, *Chenopodium*, typ *Cichorium*, *Convolvulus*, *Cornus*, *Dipsacus*, *Dracocephalum*, *Echinops*, *Echium*, Ericaceae, *Fagopyrum*, *Filipendula*, *Frangula*, *Geranium*, typ *Helianthus*, typ *Heracleum*, *Impatiens*, *Knautia*, Lamiaceae, *Lonicera*, *Lotus*, *Lythrum*, Malvaceae, *Medicago*, *Melilotus*, Onagraceae, *Phacelia*, *Pinus*, *Plantago*, *Polygonum*, *Reseda*, Rosaceae, typ *Rubus*, *Rumex*, typ *Salvia*, *Sambucus*, typ *Solidago*, *Symphytum*, typ *Taraxacum*, *Tilia*, *Trifolium pratense*, typ *Trifolium*, *Viburnum*, *Vicia*, *Viola tricolor*, *Zea mays*. W 2002 roku w odchodach trzmieli oznaczono 34 typy (Tab. 1), w 2003 roku 29 typów (Tab. 2), a w 2004 roku najmniej, bo 24 typy pyłku (Tab. 3).

Większość z oznaczonych typów pyłku należało do gatunków owadopylnych. Pewnej, niewielkiej części pyłku nie można było oznaczyć ze względu na znaczne zniszczenie ziaren w procesie trawienia. Zniszczone ziarna należały prawdopodobnie do gatunków roślin wytwarzających pyłek o najcieńszej eksynie, najbardziej podatnej na działanie warunków panujących w przewodzie pokarmowym owadów.

Na podstawie szczegółowych analiz próbek odchodów sporządzono dla każdego roku taśmę pokarmową trzmiela ziemnego (Tab. 1, 2, 3).

**Słowa kluczowe:** Trzmiel ziemny, *Bombus terrestris*, rośliny pokarmowe, analiza pyłkowa odchodów, trawienie pyłku.