Session e

ENTOMOPALYNOLOGY AND MELISSOPALYNOLOGY

Pollen and insectivorous bats

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Many nocturnal insects like the corn earworm moth (*Helicoverpa zea*) are insect pests on agricultural crops. The cost of controlling these insect pests costs farmers billions of dollars. Insectivorous bats play an essential role in keeping populations of night flying insects in check. Hundreds of insect per hour can be caught by a single bat.

Because of the bat's mobility, it is difficult to determine the locations and dispersal patterns of the bats as they feed on the nocturnal insects. Linking bat prey selection to the insect pests associated with agricultural crops is difficult and has important conservation implications.

Two insectivorous bat species, the fringed myotis (*Myotis thysanodes*) and the pallid (*Antrozous pallidus*) were examined for pollen as a control prior to research with bats in agricultural areas. We wanted to see if pollen occurred on these two insectivorous bats, and we wanted to assess the use of pollen as a marker for indicating the plant species on which the bat's insect prey were feeding.

Pollen samples were collected by dabbing the bats' head, face, and wing membranes with scotch tape, then folding the tape with the sticky sides together. Samples were acetolyzed and both scanning electron and light microscopy were used in the analyses.

A total of 74 pollen grains from four *Agave* types were found on the fringed myotis bat, and 80 pollen grains from additional *Agave* types were found on the pallid bat. This research indicates that pollen could be removed from the bats without harming the them and that pollen analysis of insectivorous bats is feasible and can be used to determine the plants on which the insect prey feed.

Because both of these bat species have been documented to land on the flowers of some *Agave* species, additional research is needed to further determine if pollen is transferred onto the bats from the prey or from flower visits. Pollen transferred from prey could serve to identify the existence and timing of certain flowering plant species and link bats foraging on insects associated with agricultural systems.

Pollen, an indication of stink bug dispersal

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Southern green stinkbugs, *Nezara viridula* (L.), are occasional insect pests in soybean, corn, cotton, sorghum, and other agricultural crops. Both the adults and nymphs damage the plants' stems, leaves, flowers, fruits, and seeds. Damage caused by stinkbug feeding includes the loss of plant fluids, the injection of destructive digestive enzymes, and the loss, deformation, and abortion of seeds and fruits. Although stink bugs are known to move from host to host, little is known about their dispersal between cropping systems. Pollen found on and in insect pests have proven to be an effective tool in determining long- and short-distance dispersal for insects. Although stink bugs are not known as nectar or pollen feeders, they are known to feed plant parts including flowers and fruits. Therefore stink bugs may become contaminated with pollen.

Adult stink bugs were collected in Burleson Co., TX and examined for pollen. Scanning and environmental scanning electron microscope analyses and light microscope analyses were conducted on these insect pests to determine the presence of pollen and or spores. Pollen and spores were found in light microscopy analyses but not in electron analyses. Seventeen pollen taxa and three spore taxa were found in the stinkbugs including pollen from Asteraceae, cotton (*Gossypium hirsutum* C. Linnaeus), corn (*Zea mays* C. Linnaeus), and false honeysuckle (*Gaura* sp.). In laboratory tests when fed 50% sugar-water containing *Lycopodium clavatum* C. Linnaeus spores, 100% of the stinkbugs contained spores for up to seven days. The presence of pollen and the longevity of the *Lycopodium* spores indicate that pollen analyses can be used to determine dispersal. However, future research is needed to correlate the pollen recovered from stink bugs and surrounding habitats and to field test the use of *Lycopodium* as an artificial maker for stink bug dispersal.

Pollen harvest features of the Central Amazonian bee *Scaptotrigona fulvicutis* (Apidae: Meliponinae), in Brazil

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Stingless bees play an essential role in the fertilization of plant species, and are the main pollinators of many plants in the Amazon region. The majority of these plants are bisexual, requiring an external agent for carrying pollen from one flower to another. According to JOHNSON & HUBBELL (1974), Meliponinae make up the greatest share of insect biomass feeding on pollen and nectar.

Surveys of the plants used as pollen and nectar sources will reveal the Meliponid's food preferences; these plant species can be used in afforestation schemes, which will benefit bee populations.

In Central Amazonia, Anacardiaceae species had their nectar collected by *Melipona seminigra merrillae* over four months, and by *Melipona rufiventris paraensis* over nine months (ABSY *et al.* 1980). Similar observations of Anacardiaceae species as sources of pollen and nectar have been reported for two Amazonian Meliponinae species (MARQUES-SOUZA *et al.* 1995).

We have studied during a twelve-month period, pollen loads transported by *Scaptotrigona fulvicutis*, collected from the workers' corbiculae right after the hive entrance closure in an area of old secondary forest mixed with some exotic fruit trees and ornamentals. The nests were introduced in old regrowth forest on the campus of INPA-Instituto Nacional de Pesquisas da Amazônia, in Manaus, Brazil, 3°08' S and 60°10' W at an altitude of 40 meters a.s.l.

The pollen samples were obtained from five bees from a nest of *Scaptotrigona fulvicutis* on alternate days, between 07:00 and 09:00 h from August 1995 to July 1996. The entrance of the hive was closed and the bees arriving with pollen loads were captured, their loads removed and stored. Bees were set free after removing the pollen loads from their corbicula.

Ten ml of acetic acid was added to each pollen sample, then left to rest for 24h. Following this period, the samples were acetolysed using Erdtman's method. After preparation, the pollen is mount in glycerin jelly on a slide and sealed with paraffin. We counted and identified about 1,000 pollen grains from each sample and expressed the results of that count in percentages.

Once the pollen grains were identified, their monthly frequency in the samples and grouping by botanical family established that Mimosaceae, Myrtaceae and Sapindaceae, were the most frequently visited. The workers harvested the pollen from 97 plant species distributed in 73 genera and 36 families, mostly: *Stryphnodendron guianense* in April (57.37%) and *Schefflera morototoni* in May (54.73%). The harvested pollen types abundance matrix showed that there was little species dissimilarity between the months, which resulted in the formation of two large groups.

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Floral visitors, floral resource constraints and pollination limitation in *Jatropha curcas* L.

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The present paper deals with the floral biology, floral visitors and pollination of Jatropha curcas L. (Euphorbiaceae) observed in a cultivated population. The plants flower during July-September. Forenoon pattern (800-1200 h) of anthesis with subsequent (830-1230 h) pollen release was noticed. The mean number of male flowers were 44±1.7 (17-105) and female flowers were 10.2±0.64 (2-19) per inflorescence. Each flowering branch bears c.3.25 (2-7) inflorescences. Each male flower produced 1617±100 (725 -2729) pollen grains with c.162 (70-275) grains per anther. The P:O ratio was c.539:1. The apolar-radiosymmetric, inaperturate pollenswere spheroidal in shape having c.94.5μ diameter and c.6.2μ thick exine with gemmate surface ornamentation.

Thestigmas become receptive 2 hrs after anthesis. Morphological differentiation of stigmas before and during receptive period was noticed. Before receptive period the stigma lobes remained unopened. During receptive period the lobes opened showing 6-furcated stigma tips, at the junction and in between of which pollen grains were found tobe adhered. Nectar secretion coincided with pollen presentation schedule and stigma receptivity. Each female flower produced higher amount (4.54±0.82 μl) of nectar than male flower (1.92±0.44 μl) in 1200 hrs. The flower fruit ratio per inflorescence was c.10:1 and approxly 50% of female flowers set fruit with c.53% (43-61) fecundityrate. The mean number of fruit per inflorescence was c.5.4±0.73 with c.32% apomixis rate and c.2:3 seed-ovuleratio. The unisexual, regular, greenish-white, slightly odorous flowers attracted the insect visitors of Hymenoptera(*Apis dorsata, A. florea, A. mellifera, Eumenes conica, and Vespa* sp) and Coleoptera (Beetles). Nectar and pollen both were chief floral rewards. Among the different insect visitors *Apis* spp were most frequent and predominant pollen vectors with regard to floral biological features and pollination syndrome.

Other floral visitors (*Eumenes, Vespa*, and beetles) were not considered as effective pollinators because the pollination syndromes of these visitors do not match with floral structure. Rather those flower visitors might be considered as nectar and pollen robbers. To know the effect of floral rewards, especially nectar, the field experiment was performed using different grades (0.3, 0.6, 0.9, 1.2, 1.5M) of sucrose syrup. It has been observed that the different grades of sucrose have an influence on behaviour, flower visits duration, pollen removal and deposition on stigmas by honeybees (*Apis* spp), the effective pollinators of *Jatropha curcas*. Significant differences of flower visits duration were obtained after sucrose treatments. In male flowers the duration of visits by *A. florea* was highest (35s) in 1.2M sucrose treated flowers followed in degree of prevalence by *A. mellifera* (30s), *A. florea* (24s in 0.9M sucrose) and *A. dorsata* (21s) compared to control (8s in *A. dorsata*; 17s in *A. florea* and 19s in *A. mellifera*). The durations of visits were found to be lowest (5s by *A. dorsata* using 0.3M sucrose, 10s by *A. florea* using 0.6M sucrose, and 12s by *A. mellifera* using 0.3M sucrose) compared to control in male flowers. The flower visits duration in sucrose treated female flowers was highest (37s) by *A. dorsata* in 1.2M sucrose followed in degree of prevalence by 34s and 30s using 1.2M and 0.9M sucrose respectively by *A. mellifera* and 28s using 1.2M sucrose by *A. florea*. The number of pollen grains transported and deposited on stigmas get affected also by sucrose treatment. Highest number of pollen grains were transported and deposited on stigmas by *A. dorsata* when flowers were treated with 0.9M sucrose, whereas, it was lowest by *A. florea* in 1.5M sucrose treated flowers compared to control.

Thus, it is assumed that male-female competition with regard to floral rewards advertisement and visitors attraction may generate some special adaptive features to overcome the barriers of primary sexual selection process and plant fitness.

This view gets support from MOLAU 1993; DAFNI & KEVAN 1996 and STRAUSS 1997.

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Multivariate analysis of the accompanying pollen for the determination of the geographical origin of honey

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Beekeepers and honey packers often declare the botanical source of honey because consumers interpretate it as an indirect indication of quality and are favourable to pay higher prices. The International Honey Commission is presently working on the establishment of quality criteria of the most important European unifloral honeys.

Generally, in Western Europe honey imported from China and Latin America has a lower price than that locally produced. Different prices persist also between countries in Europe and between geographical regions within a country. Consumers' preferences determine honey demand on the market and consequently affect the price. World-wide light honeys like orange blossom or acacia honey achieve higher prices than honey blends or other unifloral honeys. In this scenario, there is a financial interest in mislabelling honeys.

Melissopalynology and several chemical methods can be used for the characterisation of the geographical origin of the honey. Pollen specialists often certify geographical source because they have a precise knowledge of the pollen spectrum of the honey locally produced; however, this is not sufficient to avoid mislabelling. If pollen spectra of honeys produced in areas with different vegetation types are easy to distinguish, it is more complex to differentiate honeys with the same botanical origin but produced in different geographical or topographical areas. Therefore, a specific unifloral honey (orange blossom, acacia, chestnut, etc.) can be produced in numerous different geographical areas, but, at present, it is difficult to unequivocally link a honey to the harvesting territory of the bees that have produced it. The aim of our work was to test multivariate statistical analyses to find an objective method in order to establish a one-to-one correspondence between honey and its geographical origin. First attempts to use advanced statistical methods to study the geographical origin of the honeys were already reported in literature (FERRAZZI & MEDRZYCKI 2002, BATTESTI & GOEURY 1992, SANCHO et al. 1991, ORTIZ VALBUENA 1987), however at the best of our knowledge, none was applied, at least in Italy, to establish the criteria for an PGI (Protected Geographical Indication) denomination of the honey.

In our work orange blossom honey and chestnut honey produced in Southern Italy were used as study cases and data were collected in subsequent years. Pollen analyses were conducted according to Louveaux et al. (1978). Data from each pollen spectrum were used to build a matrix and subjected to multivariate analyses through the software program SYN-TAX 2000©. Both ordination (PCA) and hierarchical clustering (Cluster analysis) were performed.

In most cases, the application of these statistical tools allowed to discriminate between numerous samples of honeys produced in different geographical areas. Without multivariate analysis, the comparison of pollen spectra would have been possible only between two or few samples at time. Our results showed that multivariate analysis of the accompanying pollen is a helpful tool for an objective classification of the honeys and that the possible use of this method for the determination of the geographical origin should be further pursued.

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Palynology of monofloral *Citrus* honeys from Región de Murcia and south Alicante

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Criteria to establish monoflorality in citrus honeys are diverse. So, sáenz-laín & gómez-ferreras (2000) suggest 10% as the minimum content in citrus pollen, serra-bonvehi et al. (1987) propose 15% –criterion adopted by comunidad autónoma de la región de murcia y la junta de andalucía–, and munuera & carrión (1994) put up 25% to characterize murcian honeys. This paper tries to contribute to resolve this dilemma.

By mean of light microscopy, pollen from 103 honeys alleged as monofloral from Citrus by beekeepers had been quantitative and qualitatively studied. Samples were collected in 1997, 1998, 1999 and 2000. From those 103 samples, 54% present over 25% of Citrus pollen grains. In 70% of them pollen from Citrus represent more than 20% of the pollinic content. Only 13% of samples have less than 15% of Citrus pollen grains. As a mean, in pollinic sediment from studied honeys Citrus represent 28% of the total pollen count (including anemogamous and not nectariferous species).

Pollen grains proposed as Spanish markers are Echium (Battaglini & Riciardelli d'Albore 1972) and Hypecoum (Sala-Llinares 1988) appear in 84% and 91% of samples. Other pollen types typically present in Citrus honeys (Ricciardelli d'Albore & Vorwohl 1979, Serra-Bonvehi et al. 1987, Sala-Llinares 1988) as Diplotaxis, Cistaceae, Plantago, Taraxacum and Fabaceae can be found in 100% of samples. Quercus pollen grains are present in more than 95% of samples.

Up to 96 pollen types have been found in studied honeys, with a maximum of 35 and a minimum of 11 per sample (23 as a mean). From them, 40 can be found in more than 25% of the samples. Rest from insects and fungal spores are absent in 34% of the samples. In 60% of them appear in very low quantities but in about 6% of them insect and/or fungal spores show high concentrations.

Quantitative analyses reveal a mean pollen concentration of 8017 pollen grains per gram of honey (class II of Maurizio) but pollen concentration can exceed 21800 pollen grains per gram of honey. 63% of samples were included in class II of Maurizio, and 28% were included in class III.

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Palynology and spectrometry of monofloral honeys from Región de Murcia

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By using palynology and spectrometry 24 samples of monofloral honey have been studied: 20 qualified in origin like orange blossom monofloral honeys and 4 qualified in origin like monofloral from rosemary. From

those 20 *Citrus* honeys, 14 were obtained in bulk from beekeepers and six were bought as commercial honeys in supermarkets and were used as a reference to show differences between just harvested honeys and processed ones.

Up to 24 465 pollen grains have been count, from what 93.2% have been identified. A total of 50 different taxa from 36 botanical families have been found, with a maximum of 30 and a minimum of 14 per sample. 80% of samples correspond to Class II of Maurizio (2000 to 10 000 pollen grains per gram of honey), and 20% to Class III (10 000 to 50 000).

Pollen spectra reveal that initial denomination of honeys does not fit in with floral origin in more than half of the cases, so that were found multifloral honeys (7 samples) and monofloral honeys from *Citrus* (9 samples), *Echium* (4 samples), Brassicaceae (2 samples) and *Rosmarinus* (2 samples). Must be taken in mind that monoflorality for *Citrus* and *Rosmarinus* honeys have been considered when pollen concentration of *Citrus* and *Rosmarinus* exceed 25% and 15% respectively.

Honeys from orange blossom were characterized for *Citrus* pollen concentrations over 27% and the presence of Brassicaceae, *Echium, Cichorioideae, Calendula, Hypecoum, Lotus, Helianthemum, Prunus y Cucurbita*. In Murcian *Citrus* honeys (in bulk) percentages surpassed 29%. Rosemary honeys characterized by Rosmarinus pollen concentrations over 20% and the presence of Brassicaceae, *Prunus*, Genisteae and *Quercus*. Honeys from *Echium* had about 90% of *Echium* pollen. Several honeys could be considered as monofloral from Brassicaceae because percentages of this pollen surpassed 45% (up to 83%); *Citrus, Echium* and *Zygophyllum* were important pollen types in Brassicaceae monofloral honeys. Up to seven honeys were finally qualified as multifloral honeys wit high *Citrus* pollen concentrations (11,8% as a mean).

Murcian honeys characterized by the presence of pollen grains from *Citrus, Rosmarinus*, Brassicaceae, *Echium, Hypecoum, Genisteae, Lotus, Helianthemum*, Cichorioideae, *Prunus, Dittrichia and Calendula*. In foreign (not Murcian) honeys *Plantago, Quercus, Reseda, Rosmarinus* and *Zygophyllum* were important pollen types too. Commercial honeys had much smaller quantity of specific pollen than in bulk honeys, perhaps as a consequence of mixture of monofloral and multifloral honeys in order to obtain higher volumes of expensive honeys.

Cluster ordination was used to classify pollen samples. The technique revealed as a powerful one in order to make homogenous groups of honeys and as an interesting tool helping in discriminate between monoflorality and multiflorality in some debatable cases.

Spectrometric study (transmittance) of basic components of colour (blue, green and red) and continuous registrations for the whole visible spectrum is not reliable to determine floral origin of honeys.

Pollen analyses of *Liriodendron tulipifera* honey

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Determination of a unifloral honey type is often more difficult than expected. There are a number of variables that affect the determination of a honey's type. These include the over- and under-representation of pollen, the loss of pollen during the return flight to the hive, how the samples are processed, filtering of the honey, etc. *Liriodendron tulipifera* C. Linnaeus (tulip tree) is an early (April – May) flowering tree in the USA that produces large amounts of nectar for early season honey flows. It produces a dark amber honey with a strong flavor and is highly desirable by many consumers.

Pollen analyses were conducted on 19 honey samples collected in North Carolina, USA, that were reported by beekeepers as *Liriodendron tulipifera* honey. Ten g of honey were removed from each sample and placed into a small beaker. Two *Lycopodium clavatum* C. Linnaeus tablets were added to each beaker. Samples were processed following the alcohol dilution method. Once reduced to a pollen reside, one drop was removed, placed onto a glass slide and examined for pollen. A compound light microscope was used to count the pollen grains and *Lycopodium* spores. Pollen concentration values per 10 g of honey sample were calculated. Pollen grains were identified to the lowest possible rank (family, genus, or species). Initially 500 pollen grains per sample were going to be counted. However, after processing and preliminary analyses, it was determined that all of the samples had been filtered by the beekeepers. Since the samples were filtered, a 200-grain count was made for each sample. Floral frequency classes were calculated by totaling the number of grains of a particular taxon and dividing by the total number of grains counted within that sample.

The average pollen concentration values ranged from 3,682 (sample 17) to 165,525 (sample 7) pollen grains per 10 g of honey. The low pollen concentration values of the honey samples are most likely due to filtering which can remove pollen. A total of 108 different pollen types were found in the samples, and were identified into 34 families, 38 genera, and 31 species. The number of taxa per honey sample varied from 9 (sample 8) to 29 (samples 1 and 18). Like pollen concentration values, the number of taxa found in the samples varies according to the amount of filtering of the honey prior to analyses. No single pollen type was found in all of the samples. *Liriodendron*, *Rubus*, and *Pedicularis* were found most frequently and occurred in 13 of the 19 samples.

The main focus of this research was to examine honey that was unifloral *Liriodendron* honey. Filtration of the samples made it impossible to determine if any of the honey examined was a unifloral *Liriodendron* honey. How many pollen grains were removed by filtering is not known. Although *Liriodendron* was found in relatively high numbers in several samples, a definite determination of being unifloral cannot be made. Whether nor not *Liriodendron tulipifera* pollen is over or under represented in honey needs to be researched. Additional studies need to be conducted in which honeybees can only feed on *Liriodendron tulipifera*. This will show how much *Liriodendron* pollen occurs in a unifloral *Liriodendron* honey.

Melissopalynological studies of *Salix Aegyptica L.* in Kashmir – Himalaya

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While working on Melissopalynology of Kashmir – Himalaya the author assessed the nutritive role of pollen of Salix aegyptica L. among the bees in the region during late winters and early springs of 2002-2003. This work aims to describe the most frequent fresh pollen of Salix aegyptica L. in various samples of honey collected from different parts of Kashmir – Himalaya. Besides pollen contribution (pollen spectra) was observed during the study period. All the relevant research parameters were assessed which confirms that during this period only flowering plant in full bloom was Salix aegyptica.

Pollenological range of bee load in Forest-Steppe zone of Ukraine

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Flower pollen in pollen load is an irreplaceable protein-vitamin and lipid forage not only for bees, but also a valuable dietary and medical product for human beings. Pollens of different taxons have different chemical content. A great amount of pollen is under-utilized on various agricultural and natural fields. Thus, bee families have large opportunities while gathering pollen in natural conditions.

The Forest-Steppe natural climatic zone covers more than one third of Ukraine's territory. It accounts for about 30% of all bee families and for almost half of the national honey production. The Forest-Steppe zone is situated in the central part of Ukraine, in the middle of Europe. It is featured by patchiness of both vegetation and soil content. The floristic conditions of bee keeping in the Forest-Steppe have advantages compared with other adjacent climatic zones: a greater diversity of nectariferous and polliniferous plants grow there. Woody areas alternate with steppe ones in that zone, along with high rate of cultivated lands. The wide range of crops is supplemented by various wild grasses, which surround cultivated fields. The apiary season lasts 5-5.5 months.

The work's goal was to study the specific diversity of pollen loads gathered in different periods of the apiary season in the region of our investigations. The objects of our research are pollen-grains from bee pollen loads sampled in bee-gardens of the Forest-Steppe zone of Ukraine. Taxonomic origins of pollen loads were preliminarily determined by their color (D. Hodges, 1974), shape, and relative size. The pollen was investigated in the pollen analysis laboratory of the Austrian Bee-keeping Institute. Samples for light microscopy were prepared by the method of A.J. Louveaux, A. Maurizio, G. Vorwohl (1978). The pollen was identified using LUCIA

software developed in that Institute. The pollen-grain description was made according to L.A. Kuprianova, L.A. Alyoshina (1972), it is accompanied by photographs from NICON light microscope of the Austrian Bee-keeping Institute. Photographs made on scanning electronic microscope JSM-35C owned by the Botany Institute, the National Academy of Sciences of Ukraine, are available for some taxons as well.

It was established that in the botanical terms the registered plants belong to 45 families, 6 of which are the most valuable for flower pollen gathering. They include Rosaceae (14 species), Fabaceae (13), Asteraceae (9), Lamiaceae (6), Aceraceae (6), Borraginaceae (5), and Brassicaceae (4).

Fungi spores were very seldom found on pollen load preparations. The results obtained from the pollen analysis showed that out of the 125 plants, pollen-grains of which we succeeded to identify, 28% fell to trees, 17% to shrubs, and 56% to grassy plants. In spring the pattern of this ratio essentially changed - the shares of trees, shrubs and grasses were 48%, 26%, and 26%, respectively. In summer, bees visited mainly grassy plants (74%) for pollen gathering. Shrubs and trees accounted for 17% and 9%, correspondingly. This shift in the feed basis structure is explained by the fact that the majority of trees and shrubs finish blooming in spring and early summer. Expanses of crops become the core of the feed basis for bees in summer. Analysis of the research outcomes enabled determining plants which are the key sources of pollen (at least 20% in the bee load total amount), secondary ones (6-19%), and occasional ones (less than 5%). In spring and early summer, bees gather pollen mainly from forest plants, such as Scilla, Salix, Acer plantanoides etc.; from fruit crops, such as Cerasus vulgaris, Pyrus communis, Malus domestica, plants of forest parks, forest belts and artificial plantations of trees in recreation zones - Aesculus, Amorfa fruticosa, as well as weeds - Barbarea vulgaris, Taraxacum officinale. The secondary sources of pollen in the above-mentioned plant associations include Alnus, Pulsatilla, Quercus, Juglans, Armeniaca vulgaris, Rubus, Aegopodium, Gleditsia triacanthos, different species of the gena Acer, Pinus, etc. Such crops as Brassica napus, Fagopyrum esculentum, Onobrychis vicifolia, Trifolium pratense, Helianthus annuus, Facelia tanacetifolia, Vicia faba, Zea mays, meadow plants, for example, Vicia, Trifolium, as well as weeds - Papaver rhoeas become the key pollen sources in summer.

Drawing up an atlas of pollen-grains of melliferous plants is one of aims of this work. The obtained results characterize the bee load as a diverse product from the botanical point of view, i.e. in the chemical terms as well.