



Pollen diversity in honey collected from Lithuania's protected landscape areas

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Abstract. The aim of the present work was to describe the most unique pollen found in the honey collected in protected landscapes of Lithuania. Honey samples were collected from Lithuania's protected landscape areas to study the botanical origin of pollen. A total of 17 samples of honey were obtained from different protected geographical locations of Lithuania: Varduva Scenic Landscape Reserve, Salantai Regional Park, Žemaitija National Park, Dzūkija National Park, from the border between Poland and Lithuania in the Lazdijai District, Armona Geological Reserve, Gomerta Landscape Reserve, and Krekenava Regional Park. Botanical origin of honey was determined by the melissopalynology method. Monofloral lime honey was specific for the south (Dzūkija National Park) and east (Armona Geological Reserve) of Lithuania, where pollen of *Tilia cordata* Mill. made up 79.0% and 53.9%, respectively. Monofloral caraway honey was found in the Salantai Regional Park close to Žemaitija National Park and in the Armona Geological Reserve. In these locations *Carum carvi* L. pollen in this type of honey accounted for 50.19% and 82.8%, respectively. Honey with the highest content of *Fagopyrum esculentum* M. pollen (close to 100%) was collected from Dzūkija National Park. Monofloral honey from *Onobrychis* Mill. was characteristic mainly of the Lazdijai District. *Onobrychis* Mill. pollen accounted for 52.1–54.4% of the monofloral honey and 9.2–17.8% of the polyfloral honey. Monofloral *Frangula alnus* Mill. honey was found only in the Lazdijai District, where the dominant *F. alnus* pollen made up 46.1–52.1%. *Salix* spp. pollen was identified in all kinds of honey collected in different locations of Lithuania.

Key words: melissopalynology, monofloral honey, protected landscape.

INTRODUCTION

In Lithuania honey is produced in different regions, including national parks, covering a wide range of terrains. Landscapes vary between western, southern, eastern, and northern Lithuania. A distinctive floral association can exist in different climatic terrains within the same country (Linčius, 2011). Particular floral association is a characteristic feature of different geographical areas (Ruoff and Bogdanov, 2004). Currently high importance is attached to the regions where honey is collected from the region-specific plants and its botanical origin is conspicuous. Lithuanian beekeepers living and producing honey in national parks, nature reserves, and other protected landscape areas have the

privilege of marking their honey with the legal mark “Kokybė” (Lithuanian for quality), which is special only for their honey (Skirkevičius, 2010). In the honey from those terrains it is possible to identify area-specific pollen. Quality certificates of the tested honey are issued by the accredited laboratory in Lithuania; however, there is no accredited laboratory for the determination of honey origin in this country.

Monofloral rape honey is specific to Central Lithuania. Earlier the most abundant pollen of *Brassica napus* and *Salix caprea* were identified in the honey from Central Lithuania (Čeksterytė, 2002; Baltrušaitytė et al., 2007). Monofloral willow honey is found in Italy, rape honey in Poland and Estonia (Persano Oddo and Piro, 2004; Wróblewska and Warakomska, 2009; Kirs et al., 2011). Geographical origin of a particular kind of

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honey is determined by a melissopalynological study of its pollen spectrum (Louveaux et al., 1978).

Melliferous plants produce various shapes, sizes, and surfaces of pollen grains. Their identification is complicated, therefore samples for comparison purposes (plant pollen collected by hand) to be used as reference material should be collected. Currently, depending on the research subject, laboratories use a host of microscopy techniques to study images of pollen grains.

Pollen grains examined under microscope can be seen in a polar or equatorial view. Polar position of pollen grains has one of various shapes: circular, angular, hexagonal, lobate, or semiangular, interangular, subangular. The semi-, inter-, and sub-forms are found also for lobate forms (Faegri and Iversen, 1964; Kremp, 1965; Hesse et al., 2009). In the equatorial view, the most typical shapes are rhomboidal, apiculate, rectangular, or oval. Pollen forms also can be characterized according to measures of grains, i.e. area, length of polar axis, and equatorial diameter (Erdtman, 1952; Ricciardelli D'Albore, 1998).

The present study was aimed to describe the most unique pollen found in the honey collected in protected landscapes of Lithuania and to show digital images of pollen grains found in honey.

MATERIALS AND METHODS

Sampling areas

Seventeen samples of honey were collected from Lithuania's protected landscape areas. Sampling was performed in protected areas in different parts of the country.

North-west Lithuania: Žemaitija National Park (involved in the programme NATURA 2000, the Europe-wide network of sites tasked with the preservation of natural heritage) in the Plungė District; the Varduva Scenic Landscape Reserve in the Mažeikiai District; and the Kretinga District in the Salantai Regional Park, which is close to Žemaitija National Park. The Salantai Regional Park was founded to preserve the endangered species of plants that grow in the hillside forests and natural flood plains of old valleys.

South Lithuania: Dzūkija National Park in the Varėna District.

South-west Lithuania: Lazdijai District. Commission Implementing Regulation (EU) No. 75/2012 of 30 January 2012 entering a name in the register of protected designations of origin and protected geographical indications 'Miód z Sejneńszczyzny/Łódzieszczyzny/Seinių/Lazdijų krašto medus'. The regulation refers to Polish honey produced in Suwałki

and Seiniai counties and in Lithuania in the Lazdijai District. The term 'Lazdijų krašto medus' is related to the honey produced in the Lazdijai District. The characteristic features of this district are a short plant growing season and clean environment.

Towards the north from the centre of Lithuania: Gomerta Landscape Reserve, Radviliškis District.

East Lithuania: Armona Geological Reserve, situated on the Aukštaičiai Upland and Plateau, Ukmergė District (Vilnius County).

Middle Lithuania: Krekenava Regional Park in the Kėdainiai District.

Preparation of honey slides

A honey sample of 10 g was weighed and dissolved in distilled water (20–40°C) to the volume of 20 mL. This solution was centrifuged for 10 min at 1000g. After that the supernatant liquid was poured off. The sediment was dispersed with 20 mL of distilled water to completely dissolve the remaining sugar crystals and again centrifuged for 5 min at 1000g. The supernatant was decanted leaving only the sediment. The remaining excess liquid was taken up on absorbent paper. The sediment was collected with plastic Pasteur pipettes (volume 1 mL) and spread on a slide over an area of about 20 mm × 20 mm. The slide with the sediment of pollen was dried at 40°C on a heating plate only for the time strictly necessary to dry.

The glycerine jelly (Kaiser's Glycerol Gelatine TM Merck) was liquefied by warming it to ≤40°C (either on a heating plate or in a water bath). The cover slips (22 mm × 22 mm) were warmed on the heating plate. One drop of glycerine jelly was deposited onto the cover slip and placed on the slide very slowly to avoid air bubbles. The pollen grain exine and shape were visualized under light microscope Nikon Eclipse E600. Pollen images were taken in two positions: polar and equatorial view, at 400× magnification, focusing on pollen wall and surface sculpture.

Expression of results

About 400–500 pollen grains were counted in each sample. The frequency of pollen of each melliferous plant is expressed as percentage of the total pollen sum. Honey considered as monofloral is mainly produced from one plant species or pollen content from one plant species is predominant (constituting more than 45.0%). The pollen content of other plant species is designated as follows: secondary pollen 16–45%; important minor pollen 3–15%; minor pollen <3.0% (Louveaux et al., 1978).

Slide preparation of hand-collected pollen

Pollen collected by hand from 85 melliferous plants was prepared also for microscopy. Air-dried plant pollen was shaken from flowers, some part of it was applied on the slide and covered with a cover slip overlaid with liquefied glycerine jelly. These pollen samples are stored as reference. A catalogue of coloured digital images of Lithuanian melliferous plant pollen grains was created for comparison of images of pollen found in honey to those of known pollen collected manually (Čeksterytė, 2012).

RESULTS AND DISCUSSION

In the Varduva Scenic Landscape Reserve monofloral spring honey was collected. Pollen from *Malus domestica* accounted for 54.1% in sample I (Table 1). Pollen of *Acer platanoides* was found in the highest content (7.0%) compared to other kinds of honey. Pollen of *Rubus fruticosus* was identified only in honey sample I, where it accounted for 8.2%. Monofloral honey was produced also in the Salantai Regional Park with pollen of *Carum carvi* as dominant (50.19%) and Žemaitija National Park with *Trifolium repens* dominating (71.4%). Melissopalynological evaluation of honey pollen showed minor pollen of *Trifolium repens* (2.28%) in monofloral caraway honey (sample II) and *Carum carvi* (1.6%) in clover honey (sample IV).

In Dzūkija National Park summer honey with the highest percentage of *Tilia cordata* (79.0%, sample V) and *Fagopyrum esculentum* (up to 100.0%, sample VII) pollen was collected. The composition of honey collected in the location of Gudakaimis (sample VI) was more diverse. This honey contained *Salix caprea* (44.6%) and *Fagopyrum esculentum* (26.5%). Pollen from *Rubus idaeus*, *Frangula alnus*, and *Brassica napus* varied in the range of 3.8–7.0% and *Cerasus* pollen was present as minor (2.1%). Pollen identified in those honey samples represents this region's melliferous flora from spring to the end of summer.

Monofloral spring honey from *Salix caprea* and *Frangula alnus* was collected in the Lazdijai District. Compared to other kinds of honey tested, the honey from *Frangula alnus* was conspicuous by its highest content (46.1%) of pollen of this plant. This honey was the only one having an abundant content of *Frangula alnus* pollen. A characteristic feature of honey from the Lazdijai District is monofloral honey from *Onobrychis*, which contains dominant pollen of this plant (52.1–54.4%), and polyfloral honey with *Onobrychis* pollen as secondary or an important minor (9.2–17.8%).

Three kinds of monofloral honey, lime (*Tilia cordata*), caraway (*Carum carvi*), and orchard, were identified in the samples from the Armona Geological Reserve. The dominant pollen was from *Malus domestica* (56.9%, sample XII), *Tilia cordata* (53.9%, XIII), and *Carum carvi* (82.8%, XIV) in those unifloral honey samples. Secondary pollen from *Brassica napus* (18.4%) was identified in monofloral *Tilia cordata* honey (sample XIII) and *Salix caprea* (29.0%) in the honey from *Malus domestica* (sample XII). In the latter honey, important minors were pollen from *Frangula alnus* and *Acer platanoides*, accounting for 4.9% and 4.6%, respectively.

Pollen of *Phacelija tanacetifolia* was identified only in the honey from the Gomerta Landscape Reserve. Pollen analysis showed that the honey was produced within different periods. The pollen of *Phacelija tanacetifolia* accounted for 19.5% in sample XV, while in sample XVI its content was 4.8%. The concentration of *Brassica napus* pollen reached 72.0% in the monofloral rape honey from the same area (sample XVI). In sample XV the content of its pollen made up 36.1%.

The pollen of *Robinia pseudoacacia* is rarely found in Lithuanian honey; however, in honey sample XV it accounted for 8.5%. The presence of *Salix caprea*, *Frangula alnus*, *Rubus idaeus*, *Trifolium repens*, *Centaurea cyanus*, *Arctium tomentosum*, *Carum carvi*, and *Fagopyrum esculentum* pollen in those honey samples points to a wide diversity of melliferous plants within the area of the Gomerta Landscape Reserve.

Monofloral willow honey was collected from the Krekenava Regional Park in spring. An abundant content of pollen grains of *Salix* spp. (55.5%) was identified in this honey. Pollen from *Brassica napus* making up 31.3% was secondary and of *Malus domestica* and *Frangula alnus*, important minors, accounting for 5.7% and 3.3%, respectively.

Digital images of pollen grains of selected honeys from some parts of the microscopic view are presented in Figs 1–6. Pollen of *Trifolium repens*, *Fagopyrum esculentum*, and *Salix* spp. are shown in polar and equatorial views (Figs 1, 2, and 6, respectively); all *Tilia cordata*, *Phacelija tanacetifolia*, and *Frangula alnus* pollen in polar view (Figs 3, 5, and 6, respectively); and pollen of *Carum carvi* in equatorial view (Fig. 4). Polar and equatorial images of the same pollen are not similar. Small differences in pollen morphology cause difficulties in their identification (Sawyer, 1988; Lindbladh et al., 2002). The presence of specific blends of plant pollen in honey shows its geographical origin (Pupuleku et al., 2012). Therefore identification of pollen requires an expert person and a special methodology for the classification of pollen of plant species.

Table 1. Pollen composition in honey from Lithuania's protected landscapes

Sample No.	Village	Botanical composition of honey pollen, %
Varduva Scenic Landscape Reserve, Mažeikiai District		
I	Dumčiai	<i>Malus domestica</i> Borkh. 54.1; <i>Salix</i> spp. 16.7; <i>Brassica napus</i> L. 8.9; <i>Rubus fruticosus</i> L. 8.2; <i>Acer platanoides</i> L. 7.0; <i>Taraxacum officinale</i> L. 4.3; <i>Rubus idaeus</i> L. 0.8; honeydew 0.8
Salantai Regional Park, Kretinga District		
II	Balsišķiai	<i>Carum carvi</i> L. 50.19; <i>Malus domestica</i> Borkh. 15.21; <i>Salix</i> spp. 11.02; <i>Brassica napus</i> L. 8.74; <i>Frangula alnus</i> Mill. 4.18; <i>Trifolium repens</i> L. 2.28; <i>Taraxacum officinale</i> L. 2.66; <i>Rubus idaeus</i> L. 1.90; other 3.80
III	Balsišķiai	<i>Salix</i> spp. 27.5; <i>Taraxacum officinale</i> L. 13.6; <i>Malus domestica</i> Borkh. 7.7; <i>Brassica napus</i> L. 4.2; <i>Aesculus hippocastanum</i> L. 3.1; <i>Corylus avellana</i> L. 1.4; <i>Carum carvi</i> L. 1.4; <i>Acer platanoides</i> L. 1.1
Žemaitija National Park, Plungė District		
IV	Pučķoriai	<i>Trifolium repens</i> L. 71.4; <i>Rubus idaeus</i> L. 8.9; <i>Brassica napus</i> L. 8.0; <i>Salix</i> spp. 5.9; <i>Carum carvi</i> L. 1.6; <i>Malus domestica</i> Borkh. 1.2; <i>Centaurea cyanus</i> L. 0.9; <i>Fagopyrum esculentum</i> M. 0.6; <i>Taraxacum officinale</i> L. 0.9; <i>Frangula alnus</i> Mill. 0.6
Dzūkija National Park, Varėna District		
V	Rungė	<i>Tilia cordata</i> Mill. 79.03; <i>Brassica napus</i> L. 11.55; <i>Carum carvi</i> L. 5.47; <i>Salix</i> spp. 2.73; <i>Cerasus</i> Mill. 1.21; honeydew 2.80
VI	Gudakaimis	<i>Salix</i> spp. 44.6; <i>Fagopyrum esculentum</i> M. 26.5; <i>Rubus idaeus</i> L. 7.0; <i>Frangula alnus</i> Mill. 4.0; <i>Brassica napus</i> L. 3.8; <i>Centaurea cyanus</i> L. 2.8; <i>Cerasus</i> Mill. 2.1
VII	Zervynai	<i>Fagopyrum esculentum</i> M. 100
Lazdijai District		
VIII	Leonardavo	<i>Salix</i> spp. 63.7; <i>Onobrychis</i> Mill. 17.8; <i>Brassica napus</i> L. 8.9; <i>Taraxacum officinale</i> L. 5.9; <i>Malus domestica</i> Borkh. 2.2; <i>Melilotus</i> (L.) Mill. 1.5; honeydew 9.4
IX	Rungė	<i>Onobrychis</i> Mill. 54.4; <i>Salix</i> spp. 12.2; <i>Sinapis arvensis</i> L. 10.7; <i>Trifolium repens</i> L. 7.4; <i>Acer platanoides</i> L. 4.7; <i>Brassica napus</i> L. 4.0; <i>Pyrus</i> L. 2.7; <i>Trifolium pratense</i> L. 1.3; <i>Rubus idaeus</i> L. 1.3; <i>Melilotus</i> (L.) Mill. 1.3; honeydew 5.7
X	Avižieniai	<i>Onobrychis</i> Mill. 52.1; <i>Malus domestica</i> Borkh. 14.7; <i>Salix</i> spp. 17.2; <i>Aesculus hippocastanum</i> L. 4.9; <i>Carum carvi</i> L. 4.3; <i>Sinapis arvensis</i> L. 2.5; <i>Rubus idaeus</i> L. 1.8; <i>Acer platanoides</i> L. 1.3; <i>Frangula alnus</i> Mill. 1.2
XI	Seirijai	<i>Frangula alnus</i> Mill. 46.1; <i>Salix</i> spp. 21.1; <i>Brassica napus</i> L. 13.2; <i>Onobrychis</i> Mill. 9.2; <i>Rubus idaeus</i> L. 5.2; <i>Pyrus</i> L. 5.2; honeydew 33.9
Armona Geological Reserve, Ukmergė District		
XII	Baraučizna	<i>Malus domestica</i> Borkh. 56.9; <i>Salix</i> spp. 29.0; <i>Frangula alnus</i> Mill. 4.9; <i>Acer platanoides</i> L. 4.6; <i>Brassica napus</i> L. 2.0; <i>Taraxacum officinale</i> L. 2.0; <i>Rubus idaeus</i> L. 0.6
XIII	Baraučizna	<i>Tilia cordata</i> M. 53.9; <i>Brassica napus</i> L. 18.4; <i>Rubus idaeus</i> L. 9.8; <i>Carum carvi</i> L. 5.3; <i>Trifolium repens</i> L. 5.3; <i>Salix</i> spp. 5.0; <i>Ribes</i> L. 1.6; <i>Sinapis arvensis</i> L. 0.7
XIV	Baraučizna	<i>Carum carvi</i> L. 82.8; <i>Brassica napus</i> L. 4.6; <i>Malus domestica</i> Borkh. 4.2; <i>Salix</i> spp. 3.1; <i>Rubus idaeus</i> L. 2.2; <i>Centaurea cyanus</i> L. 1.8; <i>Frangula alnus</i> Mill. 1.2; honeydew 1.5
Gomerta Landscape Reserve, Radviliškis District		
XV	Debeikiai	<i>Brassica napus</i> L. 36.1; <i>Phacelija tanacetifolia</i> Benth. 19.5; <i>Rubus idaeus</i> L. 16.7; <i>Robinia pseudoacacia</i> L. 8.5; <i>Carum carvi</i> L. 5.7; <i>Salix</i> spp. 4.3; <i>Trifolium repens</i> L. 3.2; <i>Prunus domestica</i> L. 2.5; <i>Fagopyrum esculentum</i> M. 0.7; <i>Frangula alnus</i> Mill. 1.4; <i>Carum carvi</i> L. 0.7; <i>Centaurea cyanus</i> L. 0.7
XVI	Debeikiai	<i>Brassica napus</i> L. 72.0; <i>Salix</i> spp. 7.3; <i>Phacelija tanacetifolia</i> Benth. 4.8; <i>Arctium tomentosum</i> Mill. 4.2; <i>Trifolium repens</i> L. 3.9; <i>Rubus idaeus</i> L. 3.4; <i>Fagopyrum esculentum</i> M. 3.1; <i>Carum carvi</i> L. 0.5; <i>Malus domestica</i> Borkh. 0.8
Krekenava Regional Park, Kėdainiai District		
XVII	Užupė	<i>Salix</i> spp. 55.5; <i>Brassica napus</i> L. 31.3; <i>Malus domestica</i> Borkh. 5.7; <i>Frangula alnus</i> M. 3.3; <i>Sinapis arvensis</i> L. 1.0; <i>Taraxacum officinale</i> L. 0.3; <i>Centaurea cyanus</i> L. 0.3; <i>Tussilago farfara</i> L. 0.3; <i>Aesculus hippocastanum</i> L. 0.4; <i>Acer platanoides</i> L. 0.5; <i>Trifolium repens</i> L. 0.4; <i>Rubus idaeus</i> L. 0.9

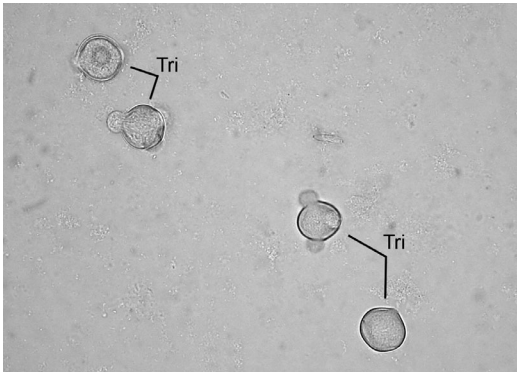


Fig. 1. Honey from Žemaitija National Park. Tri – *Trifolium repens* L.

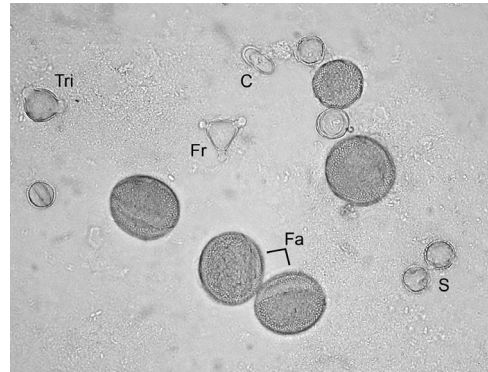


Fig. 2. Honey from Dzūkija National Park. C – *Carum carvi* L., Fa – *Fagopyrum esculentum* M., Fr – *Frangula alnus* Mill., S – *Salix* spp.

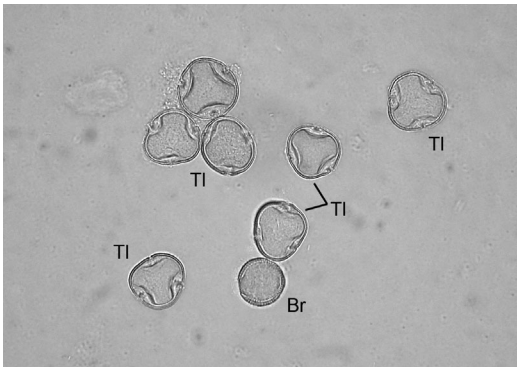


Fig. 3. Honey from the Armona Geological Reserve. Tl – *Tilia cordata* Mill., Br – *Brassica napus* L.

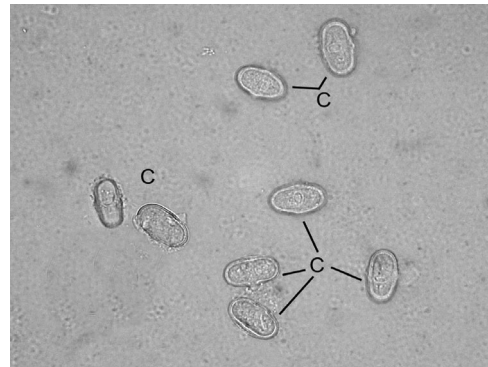


Fig. 4. Honey from the Armona Geological Reserve. C – *Carum carvi* L.

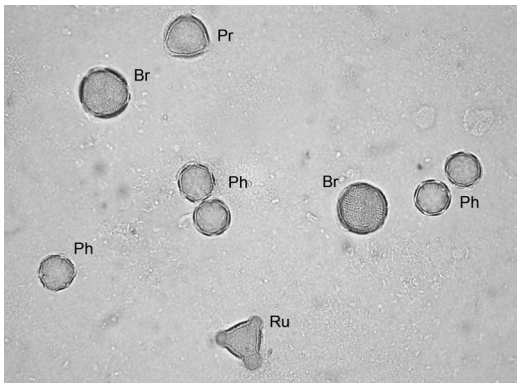


Fig. 5. Honey from the Gomerta Landscape Reserve. Ru – *Rubus idaeus* L., Ph – *Phacelija tanacetifolia* B., Pr – *Prunus domestica* L., Br – *Brassica napus* L.

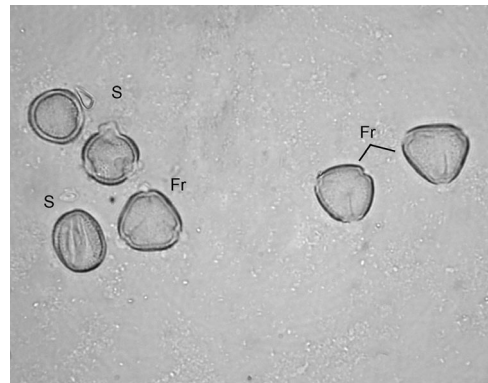


Fig. 6. Honey from Krekenava Regional Park. Fr – *Frangula alnus* M., S – *Salix* spp.

CONCLUSIONS

Lithuanian monofloral honey is produced in different locations. Buckwheat and cockshead honeys are characteristic of southern and south-western Lithuania. Buckwheat (*Fagopyrum* Mill.) is a traditional crop

cultivated in southern Lithuania and cockshead (*Onobrychis* Mill.) in the south-western areas. Production of honey with a contribution from *Phacelija tanacetifolia* Benth. and *Trifolium repens* L. is also associated with traditional farming activities in north-western Lithuania.

The data of melissopalynological analysis showed *Tilia cordata* Mill. pollen to be dominant (79.0%) in lime honey, where it was found at the highest counts in Dzūkija National Park. This kind of honey was also harvested in the Armona Geological Reserve. Natural meadow is a distinct feature of the Armona Geological Reserve and of the Salantai Regional Park located close to Žemaitija National Park. Monofloral honey was produced from *Carum carvi* L. in those terrains. Pollen of *Salix* spp. was found in monofloral and polyfloral Lithuanian honeys harvested in different terrains.

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Leedu looduskaitsealadelt kogutud mee õietolmu jaotus

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Käesoleva töö eesmärk oli kirjeldada Leedu looduskaitsealadelt kogutud mee õietolmu. Meeproovid koguti Leedu looduskaitsealadelt ja uuriti õietolmu botaanilist päritolu. Kokku koguti 17 meeproovi Varduva maastikukaitsealalt, Žemaitija ja Dzūkija Rahvusparkist, Poola-Leedu piiri äärest Ida-Suwalki järvistu alalt (Lazdijai piirkond), Gomerta maastikukaitsealalt, Krekenava parkist ning Armona geoloogiliselt kaitsealalt. Mee botaanilist päritolu mõõdeti melissopalünoloogilisel meetodil. Leedu lõuna- (Dzūkija Rahvuspark) ja idaosas (Armona geoloogiline kaitseala) domineeris monofloorne pärnemesi, *Tilia cordata* Mill. õietolmusisaldus vastavalt 79,0% ning 53,9%. Žemaitija Rahvusparkist ja Armona geoloogiliselt kaitsealalt leiti monofloorset kõõmnemett, *Carum carvi* L. õietolmusisaldus vastavalt 50,19% ning 82,8%. *Fagopyrum esculentum* M. õietolmu (peaaegu 100%) esines kõige rohkem Dzūkija Rahvusparkist kogutud mees. Lazdijai piirkonnas esines peamiselt monofloorne *Onobrychis* Mill. mesi. *Onobrychis* Mill. õietolmusisaldus oli monofloorses mees 52,1–54,4% ja polüfloorses mees 9,2–17,8%. Monofloorset *Frangula alnus* Mill. mett leiti vaid Lazdijai piirkonnast, kus domineeriv *Frangula alnus* Mill. õietolm moodustas 46,1–52,1%. *Salix* spp. õietolmu identifitseeriti kõikidest Leedu erinevatest piirkondadest kogutud mees.