

## RESULTS OF INVESTIGATIONS ON INFESTATION AND CONTAMINATION OF PROPOLIS WITH ARTHROPODS

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Received 10 October 2005; accepted 18 November 2005

### S u m m a r y

Material was collected during over 3-year studies (2003 -2005) from beehives in stationary private apiary. Propolis was scraped from various propolized hive elements, i.e. bars, frames, walls and floorboards of beehives using scraper and chisel; some samples were collected by means of special constructed propolis traps (double queen excluders installed in beehives) but a certain number of pieces was picked up also from hive debris removed from overwintered bee colonies during spring survey of beehives. Analyses of 215 samples of collected material show that 39 (18.1%) of them were free of arthropods. 81.9% were infested and contaminated with them as follows; 21.4% were contaminated in the I<sup>st</sup>, 23.3% in the II<sup>nd</sup> and 37.2% in the III<sup>rd</sup> degrees, i.e. they contained 1-2, 3-5 and over 5 pest specimens per sample (100g of propolis), respectively.

Propolis samples scraped from bars, frames and other beehive elements were contaminated in 34.1%; product collected by means of traps was comparatively clean, as only 2.9% of samples were contaminated; the strongest contamination (53.3% of samples) was stated among pieces of propolis picked up from hive debris. The commonest contaminants of propolis were insects (including dead bees and their body fragments) and mites usually accompanying bees in beehives.

Very significant components of propolis contaminations were the following insect species: wax moths - *Achroia grisella* (Fabr.), *Galleria mellonella* (L.); tenebrionid beetles - *Tribolium madens* Charp., *Tenebrio molitor* L.; dermestids - *Dermestes lardarius* L., *Dermestes maculatus* De Geer, *Anthrenus* spp.; psocids - *Lachesilla pedicularia* L., *Lepinotus inquilinus* Heyden, *Liposcelis divinatorius* Muller. The most frequent and numerous mite pests were as follows: *Acarus farris* (Oud.), *A. immobilis* Griffiths, *A. siro* L., *Tyrollichus casei* Oud., *Tyrophagus longior* (Gerv.), *T. putrescentiae* (Schr.), *Carpoglyphus lactis* (L.), *Glycyphagus domesticus* (De Geer). Sometimes they were accompanied with predators, *Cheyletus eruditus* (Schr.) and *Melichares tarsalis* (Berl.). Some samples were contaminated with parasitic bee mite, *Varroa destructor* Anderson et Trueman (dead female specimens only) and representants of other groups, e.g. mites associated with soil or plants (*Oribatida*, *Tetranychoidae*). Wingless insects and other arthropods (*Aranea*, *Collembola*, *Crustacea*, *Isopoda*) were rare observed.

**Keywords:** arthropods, contamination, insects, mites, pests, propolis.

### INTRODUCTION

Propolis was usually collected on the occasion of inspections of apiaries and examinations of hives as additional bee product. Recently the interest in this ecological product has growing tendency because of its curative features and application in medicine. Biochemical

researches show that its composition is very rich including flavonoids, organic acids, terpenes and other active substances (Bankova 2000, Bankova et al. 1983, Gisalberti 1979, Greenway et al. 1990, Marciucci 1995, Popravko, 1976, Stangaciu 1998, Walker and Crane 1987). These compounds influence

biological activity, but especially antibacterial features of propolis and cause that it belongs to very valuable natural ingredients and raw materials for preparation and production of various medicines and cosmetics (Meresta, Meresta 1985, Rybak-Chmielewska, Szczęsna 2000, Rybak-Chmielewska et al. 1991, 1992, Scheller et al. 1968, 1971). Because of these such special applications propolis should be the best quality and free of pests, products of their metabolism (body secretions, excrements, cocoons, exuvia, dead specimens and their body fragments) and other contaminations. However in practice propolis contains usually some “additions” such as small pieces of wax, pollen loads, dead bees and other arthropods, which are very attractive food for various pests of hive products. These contaminants are baits for mites and insects accompanying bees in beehives, where they usually colonize beehive debris, develop in honeycombs and infest hive products (bee bread, bee collected pollen, honey) and provisions of their hosts (Chmielewski 1998, 2002, 2004a).

Biological studies conducted on attractiveness of hive products (honey, bee bread, pollen loads) for some acaroid species fed this kind of food, show that mite development and their population increase can be very intensive (Chmielewski 1978, 1992).

The aim of presented studies is a hygienic assessment of propolis collected in beehives, as concern its contamination with arthropod-pests, with special consideration to species composition of acaro-entomofauna found in this product.

## MATERIAL AND METHODS

Three year study (2003-2005) was conducted in the Apiculture Division, Research Institute of Pomology and Floriculture (Puławy, Poland).

Propolis samples were collected from resident experimental apiary of the Institute and also from stationary private apiaries localized in Puławy (total 50 beehives). Gathering of propolis was made by means of special constructed propolis traps (double queen excluders installed in beehives) or scraped from various propolized hive elements, i.e. bars, frames, walls and floorboards of beehives using scraper and chisel during the season (from spring to autumn); a certain number of pieces of material was picked up also from hive debris collected from wintering bee colonies during spring survey of beehives (Figures 1-4).

Collected samples were analysed macroscopically (insect objects and other bigger arthropods) and microscopically (mites and small arthropods). Microscopic analyses of



**Fig. 1** - Entrance beehive bars covered (incrusted) with propolis (phot. orig.)



**Fig. 2** - Propolis sample collected by means of propolis traps (phot. orig.)



**Fig. 3** - A sample of propolis scraped from frames and other parts of beehives infested with arthropods; visible wax moth cocoons (phot. orig.)



**Fig. 4** - Stored propolis balls almost free of contaminations (phot. orig.)

collected material was made by means of stereoscopic and phase-contrast microscopes. Arthropods were prepared if necessary (microscopic slides) and identified with help of keys and descriptions in literature (Boczek 1980, Chmielewski 1998, Gołębiewska, Nawrot 1976, Gorham 1991, Hughes 1976, Smiley 1991, Zakhvatkin 1941).

Intensity of occurrence of arthropods in analysed propolis was determined using a three degree scale of contamination/infestation of samples, i.e.: I<sup>st</sup> - 1-2, II<sup>nd</sup> - 3-5 and III<sup>rd</sup> degree - >5 objects per 100 g of examined product.

Methods of these investigations were similar to those used in introductory observations and described earlier (Chmielewski 2002, 2004 b,c, 2005 a,b).

## RESULTS AND DISCUSSION

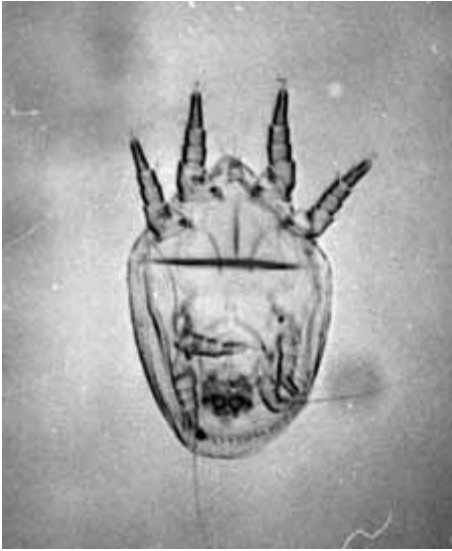
Results of analyses of 215 samples of collected material show that 81.9% of them were infested and contaminated with arthropods, mainly with insects and mites (living and dead specimens and their body fragments, exuvia, excrements, etc.). Intensity of their occurrence in propolis samples expressed in contamination/infestation degrees was diversified and depends on places and methods of their collecting (Tables 1 and 2).

Species composition of arthropods found in propolis was very varied (Table 3).

The great part of entomological contaminations were bees; majority of them were dead specimens, brood and fragments of their bodies.

Insects (*Insecta*) found in collected material were represented mainly by dermestids (*Dermestidae*), minute brown scavenger beetles (*Lathridiidae*), spider beetles (*Ptinidae*), tenebrionid beetles (*Tenebrionidae*) (imagines, larvae, excrements, exuvia), wax moths (*Pyralidae*) (caterpillars, cocoons, excrements), psocids (*Psocoptera*). Living and dead specimens, larval and imaginal forms were found.

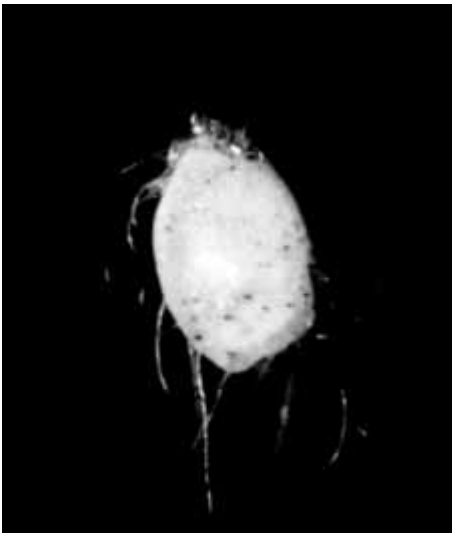
Among mites (*Acarina*), acaroid species (*Acaroiodea*) were significant part of contaminations. The most numerous of them were some species belonging to families *Acaridae*, *Carpoglyphidae* and *Glycyphagidae*. Their adults and all juvenile instars (eggs, larvae, nymphs) were stated; hypopoplar forms (hypopoplar stage or hypopodes) of these species (mainly *Glycyphagus*, *Lepidoglyphus*, *Acarus*) were also observed. Hypopoplar forms are survival forms of acaroid mites (Figures 5-7). Many representants of this mite group are commonly known as important pests of stored products, inhabitants of houses and invaders of bee nests; they



**Fig. 5** - *Acarus farris* (Oud.) - hypopus  
(author's phot. orig.)



**Fig. 6** - *Carpaglyphus lactis* (L.) - hypopus  
(author's phot. orig.)



**Fig. 7** - *Glycyphagus domesticus* (De Geer) -  
hypopus (author's phot. orig.)

infest also hive products (honey, bee collected pollen, bee bread, honeycombs) including propolis (Chmielewski 1992, 1998, 2004a).

Some samples were contaminated also with predatory mite species from families *Cheyletidae* (*Cheyletus eruditus* (Schr.)) and *Aceosejidae* (*Melichares tarsalis*

(Berl.)), which are natural enemies of acaroids and other small arthropods usually accompanying bees in their nests and infesting host's provisions.

Propolis was also often contaminated with bee mite, *Varroa destructor* Anderson et Trueman (mainly dead females), which belongs recently to the commonest parasites of honeybees. Representatives of soil mites (*Oribatida*) or pests of trees and other plants (*Tetranychoidae*) were also sometimes observed.

Other groups of arthropods (spiders, springtails, crustacean, isopodids), e.g. house (book) pseudoscorpion, *Chelifer canroides* (L.), were rare or occurred rather sporadically only (Table 2 and 3).

## CONCLUSIONS

1. Present results confirm earlier introductory observations (Chmielewski 2002, 2004b,c) that arthropods (*Arthropoda*) are significant elements of biological contaminations of propolis collected from beehives.

Table 1.

Intensity of occurrence of arthropods in analysed samples of propolis;  
contamination/infestation scale of samples (in degrees):  
I<sup>st</sup> - 1-2, II<sup>nd</sup> - 3-5, III<sup>rd</sup> - >5 objects per 100 g of the product

Propolis (method/place of collecting)	Number of samples		Infestation of samples (%)			
	Collected	Infested	Total	In degrees		
			I + II + III	I	II	III
Hive debris	92	92	100.-	11.9	34.8	53.3
Frames	88	68	77.3	29.6	13.6	34.1
Traps	35	16	45.7	25.7	17.1	2.9
Total	215	176	81.9	21.4	23.3	37.2

Table 2.

Intensity of occurrence of mites (*Acarina*) in analysed samples of propolis;  
contamination/infestation scale of samples (in degrees):  
I<sup>st</sup> - 1-2, II<sup>nd</sup> - 3-5, III<sup>rd</sup> - >5 objects per 100 g of the product

Propolis (method/place of collecting)	Number of samples		Infestation of samples (%)			
	Collected	Infested	Total	In degrees		
			I + II + III	I	II	III
Hive debris	92	89	96.7	20.7	30.4	45.6
Frames	88	29	33.0	11.4	6.8	14.8
Traps	35	4	11.4	11.4	0.0	0.0
Total	215	122	56.7	15.3	15.8	25.6

- Microscopical analyses of propolis samples show that synanthropic insects (dermestid and tenebrionid beetles, wax moths etc.) and mites (acarids, glycyphagid species) - common pests of stored food, inhabitants of houses and invaders of beehives, were also the main groups of arthropods found in majority of contaminated samples of this product; some of them, especially stored product and house dust mites, are known as allergic species and have medical and sanitary importance.
- Hygienic assessment of propolis as concern occurrence of insect and mite pests (including their survival instars, i.e. hypopodes of acaroids) in this product show that intensity of acaro-entomological contaminations depends from the methods and places of its collection.
- Product collected by means of a kind of propolis traps (double perforated queen excluders) was the best quality; over half of total number of samples collected using this method (54.3%) was free of arthropod contaminants; among contaminated samples only a few of them (2.9%) were infested in the highest (III<sup>rd</sup>) degree.
- The highest degree of contamination was stated in pieces of propolis picked up from hive debris; all

samples collected this way were contaminated with arthropods and over half of them (53.3%) was very strong infested (over 5 specimens per 100g of product).

6. Propolis scraped from frames and other elements of hives was stronger infested than the samples collected with help of propolis traps, but usually not so strong as material originated from hive debris.

Table 3.

List of arthropods (*Arthropoda*) found in collected propolis samples; total number of examined samples - 215; samples contaminated/infested - 81.9%

Insects ( <i>Insecta</i> )	Mites ( <i>Acarina</i> )
<p><u>Coleoptera:</u>  <i>Anthrenus museorum</i> (L.)  <i>Anthrenus verbasci</i> L.  <i>Dermestes lardarius</i> L.  <i>Dermestes maculatus</i> De Geer  <i>Ptinus fur</i> L.  <i>Stegobium paniceum</i> (L.)  <i>Tenebrio molitor</i> L.  <i>Tribolium madens</i> (Charpentier)  <i>Trogoderma granaria</i> Everts</p> <p><u>Hymenoptera:</u>  <i>Apis mellifera</i> L.  <i>Formica rufa</i> L.  <i>Lasius</i> spp.  <i>Paravespula germanica</i> F.</p> <p><u>Diptera:</u>  <i>Drosophila melanogaster</i> Meig.  <i>Musca domestica</i> L.</p> <p><u>Heteroptera:</u>  <i>Rhyparochromus vulgaris</i> (Schill.)</p> <p><u>Lepidoptera:</u>  <i>Achroia grisella</i> (F.)  <i>Cadra cautella</i> (Walker)  <i>Ephestia elutella</i> Hubner  <i>Galleria mellonella</i> (L.)  <i>Plodia interpunctella</i> (Hubner)  <i>Tineola biseliella</i> (Hummel)</p> <p><u>Psocoptera:</u>  <i>Lachesilla pedicularia</i> L.  <i>Lepinotus inquilinus</i> Heyden  <i>Liposcelis divinatorius</i> Müller</p> <p><u>Dermaptera:</u>  <i>Forficula auricularia</i> L.</p> <p><u>Thysanura:</u>  <i>Lepisma saccharina</i> L.</p>	<p><u>Acaridae:</u>  <i>Acarus farris</i> (Oud.)  <i>Acarus immobilis</i> Griffiths  <i>Acarus siro</i> L.  <i>Tyrolichus casei</i> Oud.  <i>Tyrophagus longior</i> (Gerv.)  <i>Tyrophagus putrescentiae</i> (Schr.)</p> <p><u>Aceosejidae:</u>  <i>Melichares tarsalis</i> (Berl.)</p> <p><u>Belbidae:</u>  <i>Belba</i> spp.</p> <p><u>Carpoglyphidae:</u>  <i>Carpoglyphus lactis</i> (L.)</p> <p><u>Cheyletidae:</u>  <i>Cheyletus eruditus</i> (Schr.)</p> <p><u>Glycyphagidae:</u>  <i>Glycyphagus domesticus</i> (De Geer)  <i>Lepidoglyphus destructor</i> (Schr.)</p> <p><u>Macrochelidae:</u>  <i>Macrocheles</i> spp.</p> <p><u>Parasitidae:</u>  <i>Parasitellus fucorum</i> (De Geer)</p> <p><u>Tarsonemidae:</u>  <i>Tarsonemus fusarii</i> Cooreman</p> <p><u>Tetranychidae:</u>  <i>Bryobia</i> spp., <i>Tetranychus</i> spp.</p> <p><u>Tydeidae:</u>  <i>Tydeus</i> spp.</p> <p><u>Varroidae:</u>  <i>Varroa destructor</i> Anderson et Trueman</p>

## ACKNOWLEDGMENTS

The author would like to thank Dr. Dariusz Teper (Apiculture Division, Research Institute of Pomology and Floriculture, Puławy, Poland) for making photos of propolis samples (Figs 1-4) for this presentation.

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## WYNIKI BADAŃ PORAŻENIA I ZANIECZYSZCZENIA PROPOLISU PRZEZ STAWONOGI

C h m i e l e w s k i   W .

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

Występowanie stawonogów w propolisie stwierdzono już w trakcie przeprowadzonych obserwacji wstępnych (1995-1999), a kontynuowane ostatnio (2003-2005) badania miały na celu poszerzenie wiedzy na ten temat i stanowią eksperymentalne potwierdzenie uzyskanych wcześniej wyników.

Próby materiału pobierano w pasiekach stacjonarnych na terenie Puław, zgodnie z ustalonym planem i opracowaną wcześniej metodyką (Chmielewski 2002). Propolis zeskrobywano z różnych elementów konstrukcyjnych uli (ramki, beleczki powałkowe, ściany, dennice) przy okazji wykonywania prac pasiecznych, w ciągu całego sezonu pszczelarskiego. Do zbierania propolisu wykorzystywano też poławiacze propolisu (tzw. „kitołapki”) w formie odpowiednio zestawionych krat odgrodowych, instalowanych w ulach między kondygnacjami gniazdowymi a nadstawkowymi. Poza tym wybierano też i wykorzystano do analiz okruchy propolisu z osypu zimowego, usuwanego z uli przy okazji wiosennego przeglądu rodzin pszczelich.

Analizy makro- i mikroskopowe 215 zebranych prób propolisu wykazały, że w większości (81.9%) z nich występowały stawonogi, głównie owady i roztocze; przy czym te ostatnie stwierdzono w ponad połowie (56.7%) przebadanych prób. Wszystkie próby propolisu wybranego z osypu gromadzącego się na dennicach uli w czasie zimowania pszczół zawierały stawonogi. Próby te w większości były silnie zanieczyszczone; w ponad połowie z nich stwierdzono najwyższy - III stopień zanieczyszczenia (ponad 5 osobników w przeliczeniu na 100g produktu). Lepszy stan higieniczny stwierdzono w przypadku propolisu zeskrobywanego z ramek i beleczek powałkowych (77.3% prób zanieczyszczonych), a stosunkowo najmniej prób (45.7%) z tego typu zanieczyszczeniami było wśród tych zebranych za pomocą poławiaczy propolisu.

Stałym niemal elementem zanieczyszczeń większości prób propolisu były martwe pszczoły i fragmenty ich ciał (odnóża i ich segmenty, skrzydła, włoski i kawałki chitynowych osłon ciała i in.). Skład gatunkowy innych stawonogów znalezionych w tym produkcie był urozmaicony, jednakże szczególnie często i licznie występowały w nim synantropijne gatunki owadów i roztoczy przechowalnianych i pasiecznych.

Spośród owadów do najczęściej spotykanych w badanym materiale należały imagines chrząszczy, ich larwy, wylinki i odchody - *Anthrenus museorum* (L.), *Dermestes lardarius* L., *Dermestes maculatus* De Geer, *Ptinus fur* L., *Tenebrio molitor* L., *Tribolium madens* Charp.; motyle reprezentowane były głównie przez barciaki (gąsienice, oprzędy, ekskrementy, fragmenty ciała imagines) *Achroia grisella* (F.) i *Galleria mellonella* (L.); częstym elementem zanieczyszczeń propolisu były także różne formy rozwojowe psotników (*Psocoptera*).

Z roztoczy do szczególnie licznie i często znajdowanych w tym produkcie należy zaliczyć rozkruszki (*Acaridae*, *Glycyphagidae*), m.in. *Acarus farris* (Oud.), *Acarus immobilis* Griffiths, *Acarus siro* L., *Carpoglyphus lactis* (L.), *Glycyphagus domesticus* (De Geer), *Lepidoglyphus destructor* (Schr.), *Tyrophagus longior* (Gerv.), *Tyrophagus putrescentiae* (Schr.). Obserwowano żywe i martwe osobniki w różnych stadiach rozwojowych i formy przetrwalnikowe (hypopusy) niektórych gatunków. W części prób zaobserwowano roztocze drapieżne: *Cheyletus eruditus* (Schr.) i *Melichares tarsalis* (Berl.), martwe pasożyty pszczoł, *Varroa destructor* Anderson et Trueman, a także niekiedy roztocze glebowe, nadrzewne, przedziorki i in. (*Oribatida*, *Tetranychoida*). Inne stawonogi (*Aranea*, *Collembola*, *Crustacea*, *Isopoda*), np. zaleszczotki, *Chelifer canroides* (L.), należały zwykle do rzadkości i były na ogół nielicznie reprezentowane.

**Słowa kluczowe:** owady, propolis, roztocze, stawonogi, szkodniki, zanieczyszczenia.