



Ricola Foundation
Nature & Culture

11th COLOSS Conference



Proceedings

Lukovica, Slovenia, 21-23th October 2015



Eva Crane Trust

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**UNIVERSITÄT
BERN**



Agricultural Institute
of Slovenia



Univerza v Ljubljani
Biotebniška fakulteta



11th COLOSS Conference

'Current threats & future beekeeping'

TOPIC

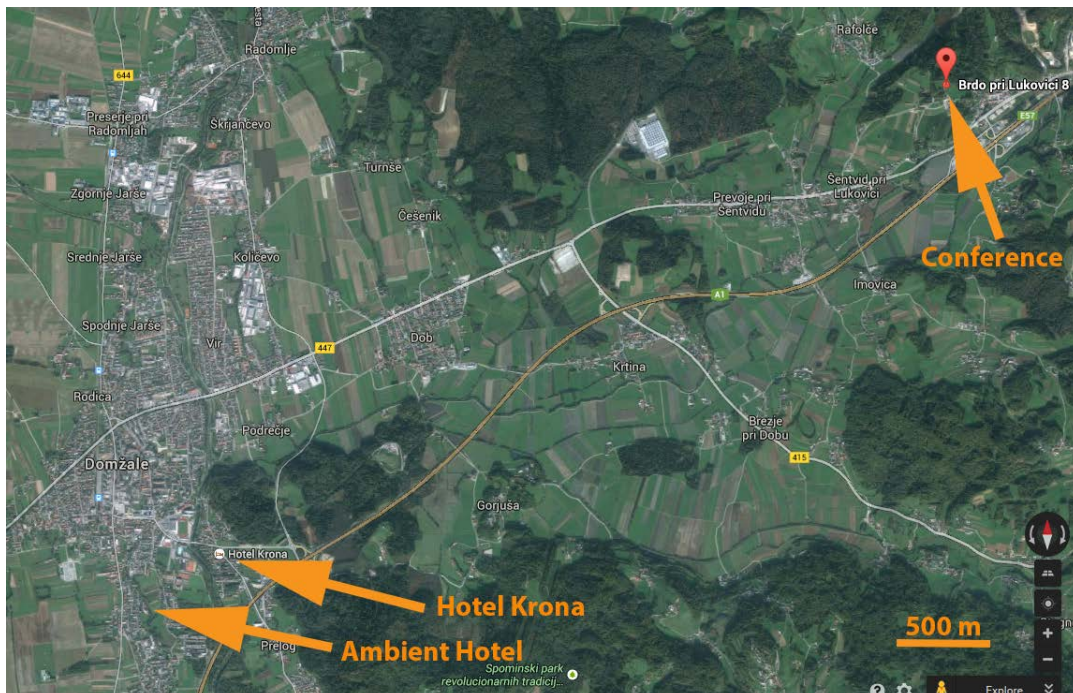
- International meeting of COLOSS to provide an update on the network's achievements and future directions, including meetings for COLOSS Core Projects, Task Forces, and sessions on small hive beetle and beekeeping.

WHEN

- 20 October** Executive Committee Meeting in evening (open to EC members only)
- 21 & 22 October** COLOSS General Assembly and discussions (open to all COLOSS registrants)
- 23 October** COLOSS discussions (open to all COLOSS registrants) & extension morning (open to all)

WHERE

- Slovenian Beekeepers' Association
Brdo pri Lukovici 8, 1225 Lukovica, Slovenia
www.czs.si ([Link](#) to Google Map)



REGISTRATION FEE

- 20 Euros payable on site (please bring correct cash).

POSTER SESSION

- **All poster abstracts have been accepted.**
- All participants submitting abstracts for posters are expected to present their posters during the evening apéro on 21 October (see schedule for details).
- Poster dimensions: no larger than **A0** (84.1x118.9 cm)

FUNDING

- Registration fee will cover all coffee breaks, lunches, and the social dinner on Wednesday.
- Due to limited financial support, participants will **NOT** be reimbursement for travel and accommodation. Further information will be provided should this reimbursement status change.

ACCOMMODATION

1. Ambient Hotel <http://www.ambienthotel.si/domov.html>

Aškerčeva ulica 6a, 1230 Domžale
+386 8 200 2000

2. Hotel Krona <http://www.hotel-krona.com>

Ihanska cesta 2, 1230 Domžale
+386 1 72 40 600

Price list with breakfast at both hotels (including VAT):

- Single room: 49,00 Euros; Double room: 68,00 Euros; Triple room : 90,00 Euros;
Tourist tax: 1,00 Euros per person per day. Payable in the hotel.

Accommodation reservations are handled by the Aritours Travel Agency. Reservation is confirmed only after payment.

Aritours Travel Agency

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Reference: API 1505 COLOSS
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TRANSPORTATION

- **Ljubljana Jože Pučnik Airport International Airport to Domžale**
 - Taxi: 17 km, 25 min., ~20 Euros
- **Ljubljana city centre to Domžale**
 - Taxi: 20 km, ~30 min., ~20 Euros
 - Bus: 1,8 € (each hour) 20 minutes (<http://www.kam-bus.si/> only in Slovenian).
 - Train: 1,85 € (each hour) 20 minutes
(<http://www.slo-zeleznice.si/en/passengers/slovenia>)

Transfer between hotels to the meeting location at the Slovenian Beekeeping Association centre will be organized and covered by registration fees and meeting support.

Agenda

20th OCTOBER 2015 - @ Ambient Hotel

Time	Session 1 – COLOSS Executive Committee Meeting 1 (for Executive Committee members only)
19:00-20:30	Meeting of the COLOSS Executive Committee

21st OCTOBER 2015 – @ Slovenian Beekeepers' Association

Time	Session 2 – COLOSS General Assembly Meeting 1
07:30	Bus departs to Slovenian Beekeepers' Association from Ambient and Krona hotels
07:45-08:30	Sign-in & coffee
08:30-08:35	Welcome by COLOSS President Peter Neumann and Local Organizing Committee Chair Peter Kozmus
08:35-09:30	General Assembly Discussions
09:30-09:45	Welcome from the Slovenian Ministry of Agriculture
09:45-10:00	Small hive beetle <i>ante portas</i> by Peter Neumann
10:00-10:15	Small hive beetle in Italy by Franco Mutinelli
10:15-10:30	<i>Vespa velutina</i> in Europe by Marco Porporato
10:30-11:00	Break , with drinks & snacks
Session 3 – COLOSS Updates	
11:00-12:30	COLOSS Core Project & Task Force updates annual achievements (3 CPs & 5 TFs; 10 mins. ea.)
12:30-14:00	Lunch (covered) & poster set-up
Session 4 – Concurrent Discussion Groups 1	
14:00-16:00	1. Monitoring, 2. B-RAP, 3. BEEBOOK
16:00-16:30	Break
Session 5 – Concurrent Discussion Groups 2	
16:30-18:30	1. CSI Pollen, 2. Small Hive Beetle, 3. Bee Breeding
Session 6 – Posters & Social Dinner	
18:30-19:30	Poster session with apéro
19:30-21:30	Social dinner (covered)
21:30	Bus departs to Ambient and Krona hotels

22nd OCTOBER 2015 – @ Slovenian Beekeepers' Association

Time		Session 7 – Concurrent Discussion Groups 3
08:00	Bus departs to Slovenian Beekeepers' Association from Ambient and Krona hotels	
08:30-10:30	1. Varroa control, 2. APITOX, 3. Monitoring	
10:30-10:45	Break, with drinks & snacks	
		Session 8 – Concurrent Discussion Groups 4
10:45-12:45	1. B-RAP, 2. Small Hive Beetle, 3. Varroa Control	
12:45-14:30	Lunch (covered)	
		Session 9 – Concurrent Discussion Groups 5
14:30-16:30	1. APITOX, 2. CSI Pollen, 3. Bee Breeding	
16:30-16:45	Short break	
		Session 9 – COLOSS General Assembly Meeting 2
16:45-17:45	Updates from Core Projects & Task Force discussions	
17:45-18:30	Final General Assembly discussions, plans & Farewell	
18:30-20:00	Dinner (on own at beekeeper association ~15 euros)	
20:00	Bus departs to Ambient and Krona hotels	
		Session 10 – Executive Committee Meeting 2 (for Executive Committee members only)
20:00-21:00	Closing meeting of the COLOSS Executive Committee	
21:00	Bus departs to Ambient and Krona hotels	

Beekeeping Extension Day – Tentative Schedule

23th OCTOBER 2015 - @ Slovenian Beekeepers' Association

Time	
08:30	Bus departs to Slovenian Beekeepers' Association from Ambient and Krona hotels
09:00-10:00	Global honeybee colony losses and mitigation: a COLOSS perspective by Peter Neumann
10:00-11:00	Small hive beetle: past experiences and future perspectives by Jamie Ellis
11:00-12:00	<i>Varroa</i> : treatment approaches by Vincent Dietemann
12:00-13:00	Wrap-up Discussion
14:00	Bus departs to Ambient and Krona hotels
Concurrent session for COLOSS registrants only	
09:00-12:00	Discussion Groups for 1. B-RAP, 2 APITOX, 3. Bee Breeding

ORGANIZER CONTACTS

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Dear colleagues,

On behalf of the organizing team, I would like to welcome you to the 11th COLOSS conference in Lukovica, Slovenia.

I would like to thank all the people, who help to organize and conduct this meeting. In particular, it would have been impossible without the tireless efforts of Dr. Peter Kozmus and his local team and the kind support of the Slovenian Ministry of Agriculture, Forestry and Food.

Appreciation is also addressed to all contributors for submitting their abstracts, which I hope will stimulate rewarding discussions on colony losses and the underlying factors and mechanisms. Please keep in mind that one focus of this meeting will be to plan our activity until the next COLOSS conference in Cluj, Rumania.

Financial support is kindly granted by the Slovenian Ministry of Agriculture, Forestry and Food; the Eva Crane Trust; the Ricola Foundation *Nature and Culture*; Veto Pharma and the Vinetum Foundation.

I am looking forward meeting all of you, and hope you will enjoy this conference.

Yours sincerely,

Peter Neumann, President COLOSS Association

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PLENARY ABSTRACTS

Small Hive Beetle *ante portas*

Registrant: Peter Neumann

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Affiliation(s):

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² University of Pretoria

The small hive beetle has been detected in Italy in 2014 and despite rigorous sanitation measures again this year. Here, I will give an overview of the current global small hive beetle situation as an example of an emerging bee health factor. Small hive beetles are generalists native to sub-Saharan Africa and reproduce in association with honeybees, bumblebees, stingless bees, fruits and meat. They have become an invasive species and introductions have been recorded from America, Australia, Europe and Asia since 1996. While SHB are usually considered a minor pest in Africa, they can cause significant damage to social bee colonies in their new ranges, thereby calling for adequate mitigation.

Early reaction measures, management and surveillance of small hive beetle in Italy

Registrant: Franco Mutinelli

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Abstract:

Aethina tumida Murray (small hive beetle, SHB) was firstly reported in Italy on 5 September 2014. Three nuclei containing honey bees (*Apis mellifera*) in a clementine (citrus) orchard near the Gioia Tauro port in the Calabria region (South-west of Italy) were heavily infested with adults and larvae. 59 SHB infested apiaries have been detected in Calabria region and one in Sicily region until December 2014. No further infested apiaries have been detected in 2015. *A. tumida* infestation is a notifiable disease of honey bees in the European Union as well as an OIE listed disease. Early reaction measures adopted in Italy require immediate notification of *A. tumida* discovery to the local veterinary services and cannot move their colonies. Furthermore, a protection area (20 km radius) and surveillance (100 km radius) zone were established. The surveillance zone includes the whole territory of Calabria region, and of Sicily region, following SHB detection in a single municipality in November 2014. Compulsory visits to all apiaries in the protection zone with georeferentiation and visual colony inspection according to 5% expected prevalence (95% CI) are applied. Destruction of infested apiaries is compulsory and the soil under the infested colonies must be ploughed and treated with pyrethroids. If apiaries in the protection zone are found to be negative, traps are placed. In the surveillance zone, apiaries are selected according to a risk analysis or randomly and colonies are inspected according to 2% expected prevalence (95% CI). No movement of colonies was allowed within the protection zone. In the surveillance zone, the movement of colonies was allowed only following two negative controls 21 days apart. No movements were allowed between protection and surveillance zone. Restriction measures to movements of at risk commodities have been adopted by EU Commission. Compensation for stamping out has been defined according to the law in force. SHB surveillance program is in progress at national level.

***Vespa velutina*: a new colony loss cause in temperate Europe**

Registrant: Marco Porporato

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Affiliate(s) :

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Vespa velutina nigrithorax Du Buysson, an invasive alien hornet from South-East China, had been reported in Europe from the Bordeaux area (France) ten years ago for the first time. Till now it has reached most of France, North Spain in 2010, the Northernmost part of Portugal in 2012, North-West Italy in 2012 and South-West Germany in 2014; in Spain, Italy, and Germany, *V. velutina* was firstly observed near the French border. Some specimens were also found in Belgium, but the species is not yet established in the country.

V. velutina prefers warm temperate climates, while cold and arid areas seem not to be suitable for it. In any case, a large part of Southern Europe is exposed to invasion by this hornet species. *V. velutina* eagerly preys on flying insects to get protein food for its larvae, but it shows a special taste for honey bee foragers that it catches in front of the hives. Everywhere it has settled, severe colony losses have been experienced by local beekeepers and both migratory beekeeping and honey production have been impaired. Therefore, *V. velutina* may be considered as a potentially major cause of colony collapse in temperate European areas.

Many efforts have been carried out in the infested areas to control this invasive species both by beekeepers and by research institutions at local, regional, and national levels. The results so far achieved can be considered at best rather partial and therefore a joint European action should be implemented to face this new threat to the European beekeeping. The involvement of the Coloss network in such an action would be highly advisable.

ABSTRACTS FOR POSTER PRESENTATIONS

Controlling *Varroa destructor* with Apivar® (Veto-Pharma): results obtained in France, Autumn 2014 in high infestation context, and comparison with efficacy tests organized by FNOSAD in different regions of France from 2007 to 2014 inclusive

(Conference Sponsor)

Registrant: Benoit Siefert

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Author(s):

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Affiliation(s):

Abstract:

A field trial was conducted in Chaillac (36310), center of France, with Apivar® for 10 weeks between 1st sept – 10 nov 2014 in 8 colonies. Control treatments were applied after treatment removal, then during broodless period. All hives were equipped with sticky board and mesh floor; varroa mites were counted regularly over all the period using the VarEval® device designed by ITSAP.

On each colony, the commercial treatment APIVAR®, 3.33% amitraz in a strip form, was evaluated by hanging two strips in the broodnest. At the end of the treatment period, challenge treatments were applied with Ectodex® (amitraz 5%), 1.25 ml dripped onto sticky board positioned over the mesh, twice at 4-day interval after strips removal, then during broodless period with Oxalic acid (5 ml of solution with 42.5g OA dihydrate in sugar syrup 1:1 dripped between frames) on 22 dec 2014. Residual varroa mites were also collected on sticky boards and counted during all the control period.

Varroa infestation levels were very high (mean 7,844 mites \pm 4,780).

Mean efficacy was 98.4% (all colonies presented an efficacy >95%) and mean residual varroa mites was 123 \pm 134 (half of the 8 colonies presented <50 residual mites after a 10-week treatment).

These satisfactory results, despite a huge initial infestation rate, are in line with those of the field efficacy tests performed annually since 2007 in France by FNOSAD, and they do not show any trend for reduced efficacy of Apivar® over now 20 years of use in French colonies.

Effect of the interaction between temperature and *Varroa destructor* on physiological performance of *Apis mellifera*.

Registrant: Patricia Aldea

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Author(s):

Patricia Aldea

Affiliation(s):

Abstract:

Apis mellifera is the most important pollinator worldwide. Today, their population densities and abundance are down worldwide, registering high mortality and economic losses. This situation has become not only an environmental problem but also economic. They described various causes of these mortalities wherein the ectoparasite *Varroa destructor* mite has been described as the main causes. This mite enters the hive and parasitic individuals and young adults, feeding on its hemolymph, producing an overall imbalance in the hive. Furthermore, climate change in different environments associated with global change, has become one of the greatest threats to biodiversity. This has had a direct impact on bees worldwide, and that has affected their physiological performance and changed in theory - interaction Varroa- bee. Due to the above, the aims of this research it is to evaluate the effect of Varroa on the physiological performance of bees when they are exposed to different environmental temperatures under laboratory conditions. It will also determine how the host-parasite changes (or not) relationship when the temperature conditions vary and the parasite load increases. Physiological measure and compare between treated and control groups, variables are: energy expenditure, thermoregulation behavior, feed consumption, mortality and half lethal temperature (TL50). The hemolymph components as vitellogenin, glycoprotein related to the health of individuals will also be assessed. It is expected that groups of healthy bees have less energy, greater capacity for thermoregulation, reduced feed intake, higher values of LT50 and hemolinfático healthy level of vitellogenin about groups of parasitized bees and that these physiological variables will deteriorate increasing the parasitic load on bees.

The Neonicotinoids Thiacloprid, Imidacloprid and Clothianidin affect the Immunocompetence of Honey Bees (*Apis mellifera* L.)

Registrant: Annely Brandt

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Abstract:

For successful beekeeping we need healthy and resilient honey bee colonies. The main thread of honey bee colonies is assumed to be the parasitic varroa mite that not only feeds on the bees but also transmits a number of harmful diseases. For the health of the honey bees, it is crucial to have strong defence mechanisms against those parasitic infections and diseases. This defence can be weakened by environmental factors like pesticides, which may leave the bees more vulnerable for parasites and pathogens.

We investigated the sublethal effects of the neonicotinoids thiacloprid, imidacloprid, and clothianidin on individual immunity, by studying three major aspects of immunocompetence in worker bees: total hemocyte number, encapsulation response, and antimicrobial activity of the hemolymph. In laboratory experiments, we found a strong impact of all three neonicotinoids. Thiacloprid (24h oral exposure, 200 µg/l or 2000 µg/l) and imidacloprid (1 µg/l and 10 µg/l) reduced hemocyte density, encapsulation response and antimicrobial activity even at field realistic concentrations. Clothianidin had an effect on these immune parameters only at higher than field realistic concentrations (50 - 200 µg/l).

These results suggest that neonicotinoids affect the individual immunocompetence of honey bees, possibly leading to an impaired disease resistance capacity. Our data will contribute to a better understanding of the complex and multi-causal colony losses and may help to establish indicators for the health status in order to avoid bee damage in practice.

Winter losses of honey bee colonies and renewal of livestock in Austria and the Czech Republic

Registrant: Robert Brodschneider

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³ Department of Protein Biochemistry and Proteomics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 27, 783 71 Olomouc, Czech Republic.

Abstract:

In Austria we have established the monitoring of honey bee colony losses during winter. Since the winter of 2007/2008 we have collected information from more than 6600 beekeeping operations regarding more than 150000 wintered colonies. Data is collected online, per mail and the survey is advertised in a beekeeping journal and at several beekeeper meetings. In particular, we maintain a website dedicated to this: www.Bienenstand.at. There is an online database, where everyone can analyze the results of past investigations. In the Czech Republic, winter losses of honey bee colonies were investigated for the second time this year. The response rate increased from 556 in the first year to 977 in the second year. In both countries, winter loss rate was much higher compared to 2013/14. In Austria, loss rate was 28.4% (95% confidence interval: 27.1-30.0) based on 1259 beekeeping operations wintering 22882 colonies. In the Czech Republic, it was 19.4% (95% CI: 17.9-21.0) based on 977 beekeeping operations wintering 19844 colonies. In both countries, queen problems were included in mortality figures. We found differences in losses between regions in both countries, ranging from 22 to 52 % in Austria, and from 11 to 32 % in the Czech Republic, respectively. In 2014, following a winter with comparably low losses in both countries, we made a follow up study to investigate renewal of livestock. We found that the number of beekeepers that breed queens or do not is quite similar between the two countries. In both countries, new honey bee livestock is rather home-grown than purchased. The main reasons for production or purchase of queens or nuclei are replacement of queens or extending the operation. However, the latter might change after a winter with high losses, where renewal of livestock is needed to compensate winter losses. We will further investigate the interplay of colony losses during winter and livestock production during summer.

The search for *Varroa* tolerance in Irish honey bees

Registrant: Keith Browne

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Abstract:

The *Apis mellifera mellifera* population in Ireland is considered as possibly one of the least genetically introgressed in the world and may contain the last genetically pure individuals. This is due to its geographical location, the low concentration of managed apiaries and a beekeeping community that generally resists the importation of honey bees. As a consequence, protection from *Varroa destructor* is extremely important, however *Varroa* was identified in the country in 1998 and is now distributed almost nationwide,.

The National University of Ireland, Galway (NUIG) in collaboration with the Native Irish Honey Bee Society (NIHBS) have initiated a breeding programme to increase the prevalence of colonies that are tolerant of *Varroa*. Preliminary data suggests that some colonies are naturally tolerant and/or have very low infestation rates. The aim is also to investigate the genetic and environmental factors that lie behind the ability of some native Irish honey bees to tolerate *Varroa* and resist diseases. The underlying premise is that phenotype and general colony health may not only be consequential on genotype but may also stem from the quality and variety of honey bee forage.

Metagenomic analysis of Varroa-infested honey bee colonies

Registrant: Nor Chejanovsky

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Author(s): Sofia Levin¹, Noa Sela² and Nor Chejanovsky¹

Affiliation(s): ¹Entomology Department and ²Department of Plant Pathology and Weed Research, Institute of plant Protection, Agricultural Research Organization, Israel

Abstract:

Varroa destructor infestation of *Apis mellifera* colonies carries and/or promotes replication of honey bee viruses. Some of them like DWV, VDV-1, KBV and IAPV have been well described and characterized but others remained unknown. To characterize better the viral population carried and exchanged between *V. destructor* and their parasitized bees we performed deep sequencing (RNA-seq) from *V. destructor* from acaricide-untreated parasitized colonies and from their corresponding honey bee hosts. Metagenomic analysis enabled identification known and unknown viruses of this host-parasite system. The identity and relevance of our findings will be presented in frame of the forthcoming COLOSS meeting.

Characterization of the Slovak bee population using the mtDNA COI–COII intergenic region

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Affiliation(s):

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Abstract:

Studies on the the COI-COII intergenic region was used to analyze population of Slovak bees. All 32 samples of mtDNA demonstrated affiliation to haplotype C, which is typical for the population of Central and Eastern Europe. Genetic methods proved presence of Carniolan subspecies of honeybee (*Apis mellifera carnica*, Pollmann, 1879) in Slovakia, with a possible drift of genes of Italian race (*Apis mellifera ligustica*, Spinola, 1806). Dawino method showed high variability within Carniolan breed from 50% to 84%, one sample belonged to Italian breed. Analyse of mtDNA showed considerable variation within each haplotype, consisted of two groups of *Apis mellifera carnica* 65% and *Apis mellifera ligustica* 35%. The group of *A. m. carnica* is represented by haplotypes C2C, C2I, C2E, C2y and C2D and the group of *A. m. ligustica* by haplotype C1a.

Jelleines – a group of peptides with antimicrobial functions with potential anti-American foulbrood properties

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Abstract:

Antimicrobial peptides are the important part of humoral immunity of animals. They occur across all organisms, including insects, where the largest number of antimicrobial peptides was isolated and characterized. This work is focused on the antimicrobial peptides present in royal jelly from honey bee (*Apis mellifera*), namely jelleine I and jelleine II. Jelleines are cleaved from the C-end of major royal jelly protein 1 (MRJP1).

We have been optimizing suitable protocols for exploring the antimicrobial activity of these peptides against various strains of *Paenibacillus larvae*, the causative agent of American foulbrood. A common diffusion assay revealed potential antimicrobial activities of jelleines against several strains of *P. larvae*. Several solutions were used for the dilution of synthetic peptides, e. g. water, MYPGP medium or 0.1% trifluoroacetic acid. Appropriate choice of diluting solution is the critical step to prevent losses of peptides on surfaces due to their high basicity. The diffusion assay does not permit to study the effect of peptides during incubation with bacteria and thus we tested luminescence or fluorescence-based assays for detailed description of anti-foulbrood activity of the peptides. These assays enable accurate quantification of live and dead cells which were incubated with antibacterial agents. This method presents opportunity to detect antimicrobial activities of jelleines and also to bring knowledge about their function in immune responses of honey bees to bacterial pathogens.

Effects of different pesticides and pathogen exposure on hemocytes in honeybee larvae (*Apis mellifera*)

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Abstract:

Honeybees (*Apis mellifera*) have developed an effective immune system, which differs significantly from the immune system of vertebrates, e.g. in the lack of antibody mediated defense. The innate immune system of insects is composed of humoral and cellular defense mechanisms. In the cellular defense mechanism, honeybees have 4 types of hemocytes within their hemolymph i.e., prohemocyte, plasmatocyte, granulocyte and oenocytoid. We evaluated whether pesticides and/or *Paenibacillus larvae* (*Pl*), a spore-forming bacterium causing American foulbrood, affect the cellular immune system. For this purpose we studied effects of different pesticides, such as dimethoate, clothianidin and fluvalinate on the total and differential hemocyte number in honeybee larvae. For dimethoate, the LD50 of adult bees, for clothianidin the 8-fold LD50 of adult bees was employed. Additionally, the influence of *Pl* spore infection, responsible for 50% mortality, on the above mentioned parameters was assessed as well as a combination *Pl*/pesticides. An *in vitro* bee larval rearing method was employed, where doses of pesticides were orally administered to the larvae within 4 days. In this treatment we observed a 50% increase in total hemocyte counts. The results point out that total hemocyte number increases highly significant under treatments with doses of dimethoate [120 ng/larva], clothianidin [32 ng/larva] and *Pl*. Our results further confirm a significant alteration of the number of differential hemocytes in pesticide-treated groups as compared to the control. This study shows that pesticide exposure has a significant effect on the recruitment of a cell-specific immune response in honeybee larvae.

***Nosema ceranae* then and now: comparing DNA sequences of this parasite between 1994 and the present.**

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Abstract:

It is thought that *Nosema ceranae* is a new threat to the European honeybee and that due to the lack of host adaptation to its presence, it can cause a very high mortality, because the bee has not had time to evolve any protective barriers. In Poland, we found *N. ceranae* in bees collected two decades ago and stored ever since. Some researchers think that the high mortality may be a result of genetic changes that this parasite might have undergone, which might have made it more virulent. In this study we attempt to compare the genetic material of *N. ceranae* obtained from 6 historical bee samples (collected in 1994 from 6 Polish apiaries and frozen ever since), with 9 bee samples collected currently, also confirmed to be infected with *N. ceranae* (also from different Polish apiaries). The DNA of the parasite will be extracted according to OIE recommendations, and the pre-sequencing PCR will be carried out using NosaRNAPol-F2 and NosaRNAPol-R2, as recommended for sequencing by Gisder et al. (2013). The product will be enzymatically cleaned and sent to an external facility for the sequencing procedure. We want to check if there is any differences between *N. ceranae* from the early 1990's and now. We hope that the next step of our research will be to investigate if the possible genetic changes in *Nosema ceranae* could influence its virulence.

Comparison of methods to assess the Varroa mite infestation on honey bees.

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Abstract:

Research were conducted in Institute of Horticulture in Pulawy according methodology developed for Coloss WG1 group. Comparison of method to asses the Varroa infestation on adult honeybees were done in two apiaries amounted 30 colonies. Apiaries were 50 km away from each other. We use fully developed colonies in Dadant hives. We realize icing sugar methods of estimation of Varroa mite and checking the method with soupy solution wash preceded by checking the natural mite fall. In order to obtain data about total varroa mite in colonies we used „short protocol” that starts at the and of June.

Differential Effects of ZnO and Ag Nanoparticles, and Diazinon on The Activity Of Membrane and Soluble Form of Acetylcholinesterase in Honey Bee Head and Thorax

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Abstract:

Honey bee is an important pollinator threatened by diverse environmental factors, potentially also by products of nanotechnologies. Deliberate application of nanopesticides will result in inputs of engineered nanoparticles into the environment, entering both soil and freshwater environments. The activity of soluble form of acetylcholinesterase (AChE) is often used as an important biomarker of neurotoxicity after exposure to xenobiotics. Recently, *in vitro* experiments suggested that the membrane form of AChE is mainly neuronal whereas the role of soluble form is largely unknown, but some suggestions of their protective role against xenobiotics have been given. Therefore, in regard to neurotoxicity, monitoring of only soluble AChE is not sufficient. In this study we investigated *in vivo* effects of ZnO NPs (500 mg/L, sucrose feeding) and Ag nanoparticles (NPs) (50 mg/L, sucrose feeding) and AChE inhibitor diazinon (1.5 mg/L, sucrose feeding) not only on the activity of soluble AChE, but also membrane AChE in honey bee head and thorax. The activity of membrane AChE in the head of untreated honey bees was much higher than the soluble confirming results of *in vitro* experiments showing that the membrane form is probably neuronal. In the thorax this ratio was much lower. The chronic 10 days exposure to ZnO NPs and Ag NPs elevated the activity of the soluble, but not the membrane AChE in the head. However, the same treatment had no effect of any form of AChE in the thorax. On contrary, the chronic exposure to AChE inhibitor diazinon diminished only the activity of soluble AChE in the head, but unexpectedly elevated the soluble AChE in the thorax. Our results indirectly confirm the difference in the function of two forms of AChE. The elevation of the activity of soluble AChE might be predictable for its detoxifying function whereas the changes in the activity of membrane AChE could be the result of the compensatory effect of nervous system or direct inhibition by diazinon. However, the role of the soluble AChE needs to be further investigated. We show that the mechanism of ZnO NPs and Ag NPs action on AChE is similar but other than the mechanism of diazinon suggesting that ZnO and Ag NPs don't act directly inhibitory on AChE at the exposure set-up (doses, and duration) used in our experiment.

Labiatae Pollen Index during the past 10 years in Spain.

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Abstract:

Given the variation in certain climatic factors, over the last decade, it has raised the approach the analysis of the representativeness of the main types of Labiadas in Spanish honeys over time. In particular, this representation is studied in the region of La Alcarria area of greater presence of the species in this large family. This study is of particular interest for the production of Spanish honeys, being one of the most recognized productions Monofloral in this country and prestige of the DOP Alcarria honey (DOP Miel de La Alcarria). The main flower honeys of the DOP Miel de La Alcarria belong to rosemary, lavender and multifloral honeys high lipped nectar composition, such as thyme, savory, among others.

With the completion of this work is to provide important data relating to changes in nutrition of bees, at the level of possible changes in Nectar which is available in recent times, in a given area.

We reviewed data from honeys produced between 2005 and 2015 and analyzed in the Honey and other Bee products Laboratory, belonging to the Agricultural Center Marchamalo-IRIAF. For this study, the results obtained from over 6000 honey samples are analyzed. A minimum of 100 honey samples from the major productions were analyzed.

Likewise, in this study, the data pollen essentially are crossed with the data of temperature and humidity for each year.

Field assessment of impacts of different neonicotinoids on honey bee queens and drones

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Abstract:

Neonicotinoids have been among the most frequently used insecticides in the cultivation of several crops and orchards. Till 2012, imidacloprid represented 41.5% of the whole neonicotinoid market and thiamethoxam was the second biggest neonicotinoid, followed by clothianidin. However, almost two years ago, a decision was made by the EC parliament to ban the use of these three compounds for two years and this will soon be re-addressed. Our aim was to determine the effect of particular neonics on: the life span of queens, effects on egg laying or brood development, sperm viability and overwintering ability when particular neonicotinoids were fed to the colonies in low and very high sub-lethal field realistic doses (e.g. 5ppb and 200 ppb respectively for imidacloprid; 20 ppb, 200 ppb and 400 ppb respectively for clothianidin); a control group was also evaluated. Additionally we looked at the colony population dynamics. The experiment was set up in different countries using the local honeybee populations and it was run during spring –summer 2013 and 2014. The first comprehensive results are presented: they show a detrimental effect of the high doses of the neonicotinoids used, while the effects of the low doses were variable and dependent on the application dose, the year and the feeding quantity of the contaminated food. Further work is needed to be done on the same direction. This research work has been undertaken by members of COLOSS honey bee research association. All authors' names, apart from the representing author, are in alphabetical order.

Eat and treat - drone brood for human consumption and *Varroa* control

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Abstract:

As global population increases, global food production is one of the major challenges for our planet, in particular animal protein. Insects include a high proportion of essential proteins, fatty acids, vitamins and minerals and they can be produced more efficient with a lower environmental impact than other livestock e.g. cows, pigs and chickens. Although insects are part of the diet in many parts of the world the interest in insects for food has only recently become relevant in the western world. Western palate simply does not know how good to eat insects can be and need to surpass the disgustingness barrier.

Honeybees are important for a sustainable food production both as primary producers of honey but in particular for their pollination service. Honeybee health is thus crucial and sustainable ways to control varroa desired. Drone removal is a sustainable method used to suppress the varroa level from spring to summer already used by many beekeepers. Drone brood is a delicious product that could as well be incorporated in our food chain. It could be a win-win situation if beekeepers could get an additional income from drone brood – thus making this sustainable varroa control methods even more appealing. In addition honeybees could with their positive brand pave the wave for insect consumption.

Case report of European foulbrood in the Czech Republic: a new outbreak after 40 years.

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Abstract:

The European countries report new cases of the European foulbrood (EFB) in honeybee (*Apis mellifera*) colonies. The disease is caused by entomopathogenic bacteria *Melissococcus plutonius*. The outbreak of EFB was recently confirmed in the Czech Republic. Based on the record it is approximately after of 4 decades of the last outbreaks in this country.

Two apiaries of one beekeeper were suffered EFB during June and August 2015. In June 2015, quick weaknesses of honeybee colonies have been recorded in the first apiary by the beekeeper. Scattered brood pattern and decreased number of honeybee larvae were observed. The melted larvae and hive bees were collected from every apiary and DNA was extracted from 10 individuals. The PCR using specific primers confirmed *M. plutonius* in larvae, open pupae and adults of *A. mellifera* in all beehives in the apiary (n=7). The second apiary is around 5 km far. Clinical symptoms were observed in 5 of 7 inspected colonies in the apiary (n=11). PCR examination confirmed *M. plutonius* in honeybee larvae and adults in colonies with clinical symptoms.

The EFB is notifiable disease in the Czech Republic. 5 km protection zone is established around apiary site with confirmed EFB. Approximately 600 – 700 colonies are present in actual protection zone. Colonies will be inspected during survey for clinical symptoms and sampled for wax debris to analyze *M. plutonius*. Results, possible source of infection and epidemiology of this outbreak will be discussed in poster at COLOSS conference in October 2015. The study is supported by the project QJ1310085 of the Czech Ministry of Agriculture.

Discrimination concentrations of acaricides for identification of *Varroa destructor* resistance *in vitro*.

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Abstract:

Although honeybee parasitic mite *V. destructor* is present in Europe around 3 decades, synthetic acaricides are widely used. We propose to test mite populations to distinguish sensitive and resistant populations to different active substance for successful treatments.

We adapted plastic vial bioassay for identification of resistant populations of *V. destructor* in the Czech Republic. The sensitivity of three different Czech varroa mites populations to tau-fluvalinate, acrinathrin and amitraz were observed in vial bioassay. The acaricidal compounds were diluted in acetone and applied to the vials. The females of varroa mites from infested larvae and pupae were added into vials. The mortality of mites was checked after 24 hours of exposition. The populations of mites differed in their sensitivity to three tested acaricidal compounds: pyrethroids acrinathrin and tau-fluvalinate; and formamidine amitraz.

Kyvalka population was resistant to acrinathrin, tau-fluvalinate and amitraz, while Postrizin were sensitive to all acaricides *in vitro*. The intermediate situation was in Prelovice population, where the mites were sensitive to acrinathrin and amitraz, but not to tau-fluvalinate. When LC₅₀ concentrations for acaricides were compared, sensitive population (Postrizin) has 85, 91 and 31 times lower concentrations than resistant population (Kyvalka) for tau-fluvalinate, acrinathrin and amitraz, respectively. The suggested discrimination concentrations are following: 1, 0.3 and 0.2 µg/mL for tau-fluvalinate, acrinathrin and amitraz, respectively. The application of discrimination concentrations could help for early detection of varroa resistant populations.

The study is supported by the project QJ1530148 of the Czech Ministry of Agriculture.

Metabolic pathway of pesticides within honeybee colonies

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Abstract:

The application of pesticides has increased massively in the last decades and their use in agricultural cropping systems is often discussed as a factor influencing bee health. Research has demonstrated that the agrochemicals can seriously affect honeybees' health and behavior. This highlights the need of multi-residue analytical methods with high analytic sensitivity in order to detect the lowest levels of contaminants in various matrices within honeybee colonies. The requirement of low LODs is related to be the toxicity of several products showing an oral and contact LD50 in the ng/g scale. Neonicotinoids are regarded as widespread threats for honeybee colonies' vitality and survival all over the world, even at sublethal doses. Therefore, we aimed at assessing the possible contamination pathway of beehives focusing our attention on Imidacloprid, studying its concentration in bees and bee products, such as pollen, honey, wax and royal jelly, which were collected from bee colonies exposed to pesticides in the field.

Colonies were placed in apple orchards in an area where Imidacloprid is widely used, and samples were collected during Spring and Summer. Pesticides residues were analyzed by HPLC-DAD and UHPLC-MS/MS. Adult bee samples showed a low contamination, with a concentration below 0.5 ng/bee. Among the different bee products, bee bread samples (collected in the combs) and pollen loads (collected with pollen traps) generally showed the highest concentration of pesticides. In all the other bee product samples, such as honey, wax and royal jelly, low levels of pesticides and in particular of imidacloprid were detected.

Pollen does not directly intoxicate foraging bees since it is carried on their external body surface. Therefore, contaminated pollen entering the beehives may represent a major route of contamination

Monitoring bee losses in Spain (2015)

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Abstract:

COLOSS Questionnaires were disseminated during 2015 to estimate the colony losses in Spain. The dissemination of the questionnaires was made by asking collaboration through beekeepers associations and during meetings. Also, some beekeepers were contacted by phone and asked to answer the questionnaire that the interviewer was filling out.

As in previous years, a low participation was achieved and only 86 beekeepers answered the questionnaire. This represented just 0.3% of participation according to the number of beekeepers in Spain. The colony mortality rate was 13.19% and the most of beekeepers declared there were no bees inside the colony (69.1% of lost colonies). Additionally, the 34.3% of the colonies were declared to be weak after winter. Conversely, only the 1.6% of the colonies were declared to have queen problems. Transhumance was an activity declared by the 29.5% of the participant beekeepers and the most of them did not have a significant flow on oil seed rape or maize (>70% in both cases). All beekeepers reported to treat against Varroa following the sanitary rules in the Country and the most used treatments were coumaphos and amitraz strips.

New methods to improve the dissemination of the questionnaire should be developed to increase the number of beekeepers collaborating in this survey.

Antennae hold a key to *Varroa*-sensitive hygiene behaviour in honey bees

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Abstract:

In honey bees, *Varroa* sensitive hygiene (VSH) behaviour, which involves the detection and removal of brood parasitised by the mite *Varroa destructor*, can actively participate in the survival of colonies facing *Varroa* outbreaks. This study investigated the mechanisms of VSH behaviour, by comparing the antennal transcriptomes of bees that do and do not perform VSH behaviour. Results indicate that antennae likely play a key role in the expression of VSH behaviour. Comparisons with the antennal transcriptome of nurse and forager bees suggest that VSH profile is more similar to that of nurse bees than foragers. Enhanced detection of certain odorants in VSH bees may be predicted from transcriptional patterns, as well as a higher metabolism and antennal motor activity. Interestingly, Deformed wing virus/*Varroa destructor* virus infections were detected in the antennae, with higher level in non-VSH bees; a putative negative impact of viral infection on bees' ability to display VSH behaviour is proposed. These results bring new perspectives to the understanding of VSH behaviour and the evolution of collective defence by focusing attention on the importance of the peripheral nervous system. In addition, such data might be useful for promoting marker-assisted selection of honey bees that can survive *Varroa* infestations.

Gene expression in Carniolan honey bee (*Apis mellifera carnica*) exposed to pesticides

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Abstract:

The Carniolan honey bee, *Apis mellifera carnica*, is important pollinator to diverse agricultural crop plants in Slovenia. Intensive agriculture depends on the use of plant protection products that can have negative effects even on non-target organisms, including honey bees. Since 2006 losses of honey bee colonies all over the world concern beekeepers and agriculture producers. Pathogens, parasitic mites and exposure to pesticides have been investigated as potential causes of honey bee death. There are three main routes of honey bee exposure to pesticide: (i) exposure from residues in nectar and pollen in the flowers of treated plants; (ii) exposure from dust produced during the sowing of treated seeds or application of granules; (iii) and exposure from residues in guttation fluid produced by treated plants. Moreover, pesticide could have an impact via pollen consumption not only on foraging honey bees but also on entire honey bee colony. The role of pesticide and their sub-lethal doses are subjects of an increasing number of studies. It is known that prolonged exposure to pesticides weaken the immune defence mechanisms of bees and increase sensitivity to certain chemicals. Separate pesticide could not be highly toxic to bees but combination of two or even more of different pesticides might lead to higher honey bee sensitivity or mortality. In our study we investigated the molecular response of honey bee workers exposed (a) to the insecticide thiamethoxam and the fungicide mancozeb and (b) to the acaricide coumaphos and the fungicide prochloraz. Expression of immune-, development- and detoxification-related genes was examined by quantitative RT-PCR. Negative impacts of pesticides and/or their combinations differed between each treatment. All combinations of pesticides up-regulated the expression of majority of immune-related genes. The expression of development-related genes (*hexamerin 70B* and *vitellogenin*) was down-regulated in honey bees treated with thiamethoxam and mancozeb, while in the expression of *hexamerin 70B* was up-regulated in coumaphos and prochloraz treated honey bees. Some of the detoxification-related genes were up-regulated and some of them were down-regulated, depending on pesticides that were used. Our results clearly show the negative effect of pesticides on honey bees at the molecular level.

Natural mating of *Apis mellifera mellifera* honey bee queens in different periods of the season

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Abstract:

Honey bee queens mate with drones in midair and controlling the process is difficult. We decided to measure some factors of natural mating influencing later quality of bee queens. Observed factors were: age of queens when performed first orientation and mating flight, number of flights, age of queens when started laying eggs, number of spermatozoa in the queens spermathecae.

At the first period of observation (queens emergence on 26th of May) number of introduced queens was 27 of which 12 successfully mated and laid eggs. They started flights at the age of 5 days and performed 1 to 4 orientation flights. The queens mated at the age of 7 days, and started oviposition when 11.5 days old on average. At the second period (queens emergence on 17th of June) number of introduced queens was 26 of which 17 successfully mated and laid eggs. The queens started orientation flights at the age of 7 days and performed up to 5 orientation flights. They mated when 9 days old and started oviposition when 14 days old on average.

Additionally some of laying eggs queens were killed to check spermathecae filling. The number of spermatozoa in spermathecae of queens was from 2.875 to 5.250 mln in the first observation period and from 2.300 to 5.550 mln in the second period of observation.

The nightmare before Christmas: first cases of thymol resistance in *Varroa destructor*

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Abstract:

Thymol mechanism of action in *Varroa destructor* is basically neurological and is related to the interference with tyramine and octopamine receptors of the nervous system of the mite. The neurological receptors used by thymol are the same used by amitraz, against which notoriously *Varroa destructor* already showed documented resistance.

In our poster we report the results of 4 field trials realized in Italy in 2008 and in 2013, to verify the miticide efficacy of two commercial thymol-based veterinary products: Apiguard[®] and ApiLife Var[®]. To evaluate the acaricide efficacy we counted the number of mites fallen on the bottom trays provided with sticky boards, during the 28 days of products administration (Apiguard[®]: 14 days for each of the two trays; ApiLife Var[®]: 7 days for each of the 4 tablets), according to the label indications. We compared the amount of mite fall due to the treatments, with the amount of surviving mites counted for one week after the application, in brood absence obtained by caging the queen for 21 days, of a double dose (4 strips/hive) of Apistan[®] (tau-fluvalinate) associated to a single dose of trickled oxalic acid solution administered at a rate of 5 ml of solution for each intercomb occupied by bees.

In the 2008 field trials, the mean acaricide efficacy we obtained with Apiguard[®] was of 72.2%±17.7, while in 2013 we reached an acaricide efficacy with Apiguard[®] ranging from 58.0%±11.3 to 61.5%±10.7. ApiLife Var[®], similarly, reached a miticide efficacy of 30,1%±5.0. In our poster, results of the recorded temperatures will be detailed.

Data obtained from the field trials above mentioned could represent the first signs of thymol resistance in *Varroa*.

Results of CSI Pollen in Cordoba (Spain) for 2015: Urban Apiary the best.

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Abstract:

The study was conducted in the municipality of Córdoba in southern Spain. The town lies in the valley of the Guadalquivir River between the mountains of the Sierra Morena (to the north) and the Guadalquivir Valley (to the south). The municipality is 1255.2 km² in area with a population of 328,547 inhabitants in 2010.

Biomonitoring stations installed at five sites were used to assess environmental quality (heavy metal pollution, pesticides and solitary bees) since 2007 to 2011. Now, CSI Pollen project was conducted since April to September in 2015 at the same five sites:

Station 1 was located west of Córdoba in a plot at the Agricultural Experimentation Centre (IFAPA). Proximity to the Guadalquivir River allowed the honeybees to access riparian vegetation.

Station S2 was installed on agricultural land approximately 15 km south of the city. The landscape is without natural vegetation and has been parcelled into large farms of predominantly cereal and sunflower crops; uncultivated space is practically non-existent.

Station S3 was in Sierra Morena, 8 km north of the city at an altitude of 577 m. The study area was predominantly Mediterranean forest, and a nearby recreational park was included.

Station S4 was located in downtown Córdoba on the rooftop of an historic monument, "Malmuerta Tower". Ornamental plants in gardens and on streets were the most accessible food sources for the bees.

Station S5 was located east of the city in an abandoned quarry with Mediterranean vegetation but few trees.

The results show us that in Station 4 (urban apiary), the quantity and variability of pollen colours were greater.

Summer in Córdoba (Spain): natural period of “brood interruption” for the control of Varroa infestations.

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Abstract:

Córdoba usually reaches very high temperatures for Summer. Temperature average higher than 40°C was reached for July 2015. Majority of colonies were with brood interruption for August. Then treatments with commercial products based on oxalic acid (Ecoxal®) and amitraz (Amitraz®) were applied. The results will be presented and discussed. Summer in South European Countries can be considered as Winter in North European Countries and an opportunity to treat against Varroa for natural period of brood interruption.

Why Researchers Do Not Listen and Beekeepers Can Not Read Maps? Why We Are So Different and What To Do To Take Good

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Abstract:

“To show his love for her, he climbed the steep mountain, he delved into the deepest ocean and crossed from one end to the widest desert in the world. She decided to leave him because he was never home”. (He is a researcher and she is a beekeeper). Adapted from Allan and Barbara Pease.

I have worked for over 20 years in development and beekeeping programs in Bolivia and Spain. As technician, researcher and beekeeper. The following comments are about that experience.

Starting point: two different worlds required to understand. Researchers generate knowledge, and tries to answer why. It does not always offer solutions and practical applications. Their main interest is the publication in impact journals. Largely influenced by the funding, public or private. Producers are very focused on solutions and practical applications. Find answers to how, when and where. Their purpose is to produce and market. It is largely influenced by social organization and commercial structure of the sector.

In the same way, the health in beekeeping has five levels of defense or actions: bees, beekeeper, veterinarian / beekeeping technician, lab of diagnosis and researching. From all these, the veterinarian / beekeeping technician has a close knowledge about the actual management (historical and clinical data) that beekeepers conducted on bee colonies locally. The lab of diagnosis, in this sense, would be a tool for the veterinary assistant in doubtful cases and researching would be an attempt to solve new problems and establishing new protocols.

Therefore, it is proposed to strengthen the function of vets and technicians in research and development projects. They can be as a bridge, to get two-way connection and translation between these two paradigms. In this sense, several examples can be considered.

Composition of hemocytes and catalase activity in Honey bee (*Apis mellifera* L.) under during wintering

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Abstract:

In the recent years, the number of honeybee colonies are decreasing worldwide especially after the winter season. Winter resistance of bees represents a complex of cellular and metabolic mechanisms. Particularly, activities of the immune and antioxidative systems play a key role in the organism protection against biotic and abiotic stresses. In insects, the success of the immune response depends on the composition of hemocytes, which vary with age, season and health condition of bees. Also, detoxification of reactive oxygen species (ROS), which are generated in excessive amounts upon stress, represents an important component of the protective mechanisms. The turnover of ROS is regulated by the antioxidative system, which includes numerous enzymes. Among them, catalase (CAT) represents the main enzyme, which breaks down hydrogen peroxide. Accordingly, the aim of our study was to evaluate the cellular composition of hemolymph and the activity of catalase in honeybees cultivated in Chernivtsi region of Ukraine under wintering conditions.

In winter (December – February) the hives were placed in a room with a constant temperature of 2°C. The temperature within the hives was 4°C. For experiments, 100-, 120-, 140- and 155-day-old worker bees were used. Composition of haemocytes was evaluated microscopically after a Giemsa staining. Activity of CAT was measured according to Aebi in (i) hemolymph and in tissues of (ii) head and (iii) thorax.

It was found that hemolymph [A1] of the studied bees contains prohaemocytes, oval plasmatocytes, spindle-shaped plasmatocytes, permeabilized cells, plasmatocytes with filopodia and oenocytoids. In haemolymph of 120-day-old worker bees the content of plasmatocytes with filopodia decreased compared to 100-day-old bees; in 140-day-old bees the content of prohaemocytes significantly increased, the content of spindle-shaped plasmatocytes decreased, whereas no essential changes were observed for permeabilized cells. Generally, the most essential changes in cellular composition of hemolymph were discovered in 140-day-old bees.

In the heads of 120- and 155-day-old and the toraces of 155-day-old bees the activity of CAT increased twofold as compared to 100-day-old bees. In contrast, the activity of CAT in the hemolymph did not change significantly during our experiment. Hence, changes of CAT activity in winter demonstrate a tissue-specific activity pattern.

[A1]hEmocytes

Concentrations of total proteins and glucose in brood of honeybee colonies fed with food additives

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Abstract:

Honeybee colonies are exposed to numerous unfavourable threats from various diseases and environmental factors. Nosemosis type C is primarily a disease of the digestive tract in adult honey bees caused by the microsporidian *Nosema ceranae*. Since there is no authorized veterinary medicinal product for the treatment of the same and antibiotics are currently forbidden throughout the European Union due to possible determination residue levels in honey, it is important to find alternative treatments to control Nosemosis type C. The food supplements are fed to honey bees to supply the nutritive requirements of colonies in areas and during periods when natural food sources (pollen, nectar or honey) are inadequate or not available and they can improve the body functions, digestion, and help better utilization of food and development of their immune system.

The aim of this study is to examine the effect of repeated applications of various food supplements (including herbs, minerals and protein ingredients) on the biochemical and histochemical indicators, including concentrations of total proteins and glucose. Mentioned parameters were determined in five day old larvae samples originated from colonies repeatedly fed with different food additives (Nozevit, NozevitPlus, Eko ZeoPet, BeeWell AminoPlus, BPC-157). Additional feeding was performed according to manufacturer's instructions for use, respectively, and for Eko ZeoPet and BPC-157 was recalculate on the basis of described use in human and veterinary medicine. Sampling were performed 10th, 20th and 30th day from initial feeding. Total protein concentrations were measured using Bradford protein assay [spectroscopic](#) analytical procedure in suspension of larvae tissue. Glucose was measured using commercial Glucose (GO) Assay Kit. For the purpose of describing glycogen and/or oxidable diols content we used the Periodic Acid-Schiff Reaction (PAS). Control group of honeybee colonies were fed with sugar syrup without any food supplement.

Results for total proteins mean values concentrations ranges as follows: Nozevit 0.087-0.120; NozevitPlus 0.094-0.137; Eko ZeoPet 0.088-0.120; BeeWell Aminoplus 0.087-0.114; and BPC-157 0.091-0.114 (mg/mg larvae tissue), respectively. Among these results and also glucose concentrations isn't found statistically significant difference, as well as between experimental and control group of honeybee colonies.

During the winter of 2014/2015, in Poland, honey bee colony losses were again high

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Abstract:

In Poland, in 2015, the survey of winter honey bee colony losses was based on the stratified randomised sampling done in 2014, so the COLOSS questionnaire was sent to the same beekeepers as in the previous year. Additional new beekeepers replaced those who, after 2014, were removed from our list for various reasons (beekeeper died, apiary closed down, wrong address).

At the beginning of April 2015, 1552 questionnaires (together with envelopes with return address and stamps) were posted to beekeepers.

By the end of June we received 469 filled in questionnaires and 94 return letters with questionnaires which, for various reasons, had not been delivered to the recipients. At the beginning of June we posted 94 letters to new randomly selected addresses and 989 reminders to nonresponders. By July 15th we received 717 filled in questionnaires in total.

The analysis of the data showed that the losses experienced by beekeepers, although low in the previous winter (7.7%), this winter were high again and reached 16.3% (overall proportion of colonies lost). The highest losses were in Kuyavian-Pomeranian province (27.8%) and in Lesser Poland (27.5%). In four other of the sixteen Polish provinces (Lower Silesian, Lubusz, Greater Poland, Opole) the losses exceeded 20%. The lowest losses were in Subcarpathia (9.8%) and West-Pomerania (10.2%). Generally the losses were the highest in the south-western regions of the country. Losses resulting from unsolvable problems with queens, revealed after winter, reached 3.6% and were a bit higher than reported in Europe (about 3.0%). The average loss experienced by Polish beekeepers was 19.4%. Unexpectedly, there were much fewer beekeepers reporting losses between 10% and 20% (152) than beekeepers reporting lower and higher losses (331 and 230 respectively). As in previous years beekeepers whose bees foraged on maize lost many more colonies than beekeepers who claimed that their bees did not have access to such plantations (20.6% and 15.5% respectively). However, in comparison to the beekeeping season of 2014, in 2015 fewer beekeepers (15% and 7.8% respectively) noticed in their apiaries symptoms which could suggest bee poisoning (the answer to the additional question in the COLOSS questionnaire). In the face of the ban on using neonicotinoids for protection of plants utilised by bees, these last results could be a contribution to the discussion on the role of neonicotinoids in summer and winter bee losses.

Citizen Scientist Investigation on pollen diversity forage available to honey bees: Italian case studies

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Abstract:

CSI Pollen is a COLOSS Task Force which aim is to investigate pollen diversity forage available to honey bees across Europe, with 18 countries participating. Pollen diversity is essential to guarantee a balanced diet for honey bees, that includes all essential amino acids and proteins, fatty acids and minerals. In fact, low pollen diversity could compromise colony health. Furthermore, we consider that the essential goals of CSI Pollen are to: 1) raise awareness among beekeepers on the importance of pollen diversity for an healthy honey bee colony, and 2) reduce the distance between honey bee research (i.e. academy) and beekeeping.

In Italy, more than 90 colonies owned by Citizen Scientist from 12 regions are available for the project. Individual beekeepers, families and school classes sample pollen pellets with pollen traps every 3 weeks for a total of 9 times per year, from April to September. First level of investigation consists in counting the different colors of pollen within each sample, while the second level of investigation consists in the palynological analysis of the samples. The specific protocol followed is available in the CRA-API website (http://api.entecra.it/immagini/CSIPollenManual_ITA_final_2015.pdf, Italian language). Two grams of each pollen sample were diluted in 10 ml of distilled water and then 10 µl of this solution were mounted on slides and used for palynological analysis. One thousand pollen grains were defined per each pollen sample.

A case study of 3 apiaries participating to CSI Italy in 2015 is presented. The apiaries are located in Lavis (Trento, Trentino Alto Adige), Terzolas (Trento, Trentino Alto Adige) and Schio (Vicenza, Veneto). First and second level of investigation were carried out on 3 colonies per apiary for each sampling date. Since the CSI Pollen Project is still ongoing, only the results concerning the data collected in the first half of the 2015 season (first 5 sampling) are analyzed.

Origin of *Apis Mellifera* in West Ukraine: a Morphological and Molecular Approach

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Abstract:

The cultivated species *Apis mellifera* includes 26 subspecies, which originated from Europe, Africa and Asia, but are widely spread around the world today. The flow of genes between the subspecies was sometimes interrupted resulting in the formation of geographical races, which are adapted to specific geographic areas. It is accepted that the West Ukraine represents an area of natural spread of three subspecies of *Apis mellifera*, i.e. *mellifera*, *carnica* and *macedonica*. However, the modern diversity of honey bees can also be attributed by artificial introduction of other subspecies.

Using morphometric measurements, we have determined the current race composition of *A. mellifera* in some apiaries of Ivano-Frankivsk and Chernivtsi regions. The following exterior features were evaluated: cubital index, discoid shift, proboscis length and the shape of the rear edge of the wax mirrors. Analysis of the collected data showed that the morphological traits of bees in the studied apiaries do not fully comply with the existing breeding standard, which could have resulted from the hybridization with bee races introduced from other regions of Ukraine.

Due to high polymorphism of the existing races and subspecies of honeybee, their intraspecific taxonomy that relied only on morphological and anatomical data remained imperfect for several decades. Accordingly, application of molecular markers (e.g., sequencing of mitochondrial loci *CoxI*, *CoxII*, etc.) is required for reliable identification of subspecies and races. Accordingly, in order to elucidate the genetic origin and to identify honeybee subspecies currently present on the territory of the West Ukraine, we amplified by PCR and sequenced a fragment of *CoxI* from several individuals. The obtained data were compared with homologous sequences available in Genbank. It was found that the analyzed individuals occupy an isolated position on the obtained phylodendrogram, demonstrating the highest similarity to *Apis mellifera ligustica*. Therefore, the data show that this subspecies could participate in formation of modern bee races distributed in West Ukraine. More sequence information from bees collected in different regions of Ukraine is required to describe their origin and evolution in more details.

SUBMITTED ABSTRACTS

Effects of Environmentally-Relevant Mixtures of Four Common Organophosphorus Insecticides on the Honey Bee (*Apis mellifera* L.)

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Abstract:

We assessed whether exposure to environmentally-relevant mixtures of four organophosphorus insecticides (OPs) exerted adverse effects on honey bees. Adult and worker bees were orally exposed for five days under laboratory conditions to mixtures of diazinon, malathion, profenofos and chlorpyrifos. Concentration in the mixtures tested were equivalent to the median and 95th centile concentrations of the OPs in honey, as reported in the literature. Effects on survival, behavior, activity of acetylcholinesterase (AChE), and expression of genes important in detoxification of xenobiotics and immune response were examined. Survival of worker bees was not affected by exposure to median or 95th centile concentrations of the OPs. Activity of AChE was significantly greater in worker bees exposed to the 95th centile concentration mixture of OPs compared to the median concentration mixture. Expression of genes involved in detoxification of xenobiotics was not affected by treatment, but the abundance of transcripts of the antimicrobial peptide hymenoptaecin was significantly greater in worker honey bees exposed to the median concentration mixture. Results suggest that short-term exposure to environmentally relevant concentrations of a mixture of OPs do not adversely affect worker honey bees.

Ukrainian Beehive Bdzhilnyk

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Abstract:

In Ukraine a new type of a beehive called "Ukrainian bdzhilnyk" was created and patented. It consists of a small brood box with 9 - 11 low-wide frames (for example, Dadan type) and a bigger honey super for 14 - 21 frames of the same size. The main peculiarity of this beehive is that the smaller brood box is put inside of a bigger honey super in cold weather on the matryoshka-based principle. The brood box with the upper side slats that serve as hand grips is covered by the plastic sheeting and the top feeder. So the bees' nest is protected with double walls, filled with air in between, besides they don't have any connection elements. Thus, with this structure we achieve better protection of bee families from cold, wind, draughts, noise intrusions, humidity changes, sunshine, etc. That's why, the bees can last through the winter very well, they use food economically and develop intensively in spring.

As soon as the brood box is full, it's installed on the upper side slats in the opening of the bottom of the bigger honey super and thereafter, depending on the weather conditions and intensity receipt nectar, you can add the necessary number of frames into this bigger honey super. The brood box in case of the necessity can be easily removed through the bee-entrance opening. In such a way it's easier to take care of a bee family and to observe its activity. The brood box from the top can be separated by the queen excluder and from the bottom by ventilation grilles. In this position the brood box remains in the shade, it is protected from direct sunlight and is well ventilated. This reduces the threat of swarming.

During the harvest period one or two small supers with 9 - 11 frames (same as the brood box) can be installed above bigger honey super. In this way 2 bee-entrances will be formed.

The author has received two patents for the beehive in Ukraine and has submitted international application No. WO 2012/177231 (published 27.12.2012).

Qualitative and quantitative aspects of sunflower pollen in relation to genetic material used

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Key words: pollen, sunflower, hybrids, self-autofertility

Abstract:

Current sunflower hybrids have a considerable genetic variation in terms of self-fertility, and it is obvious that breeders brought significant changes, even radical, to morphological and functional characteristics of the tubular flowers from sunflower head, including pollen also. In addition to the fundamental role that pollen plays in the life of sunflower plant, pollen plays some importance in the nutrition of honeybees taking into account that sunflower is the main melliferous plant in the summer.

The samples of pollen were taken from five sunflower hybrids, respectively Favorit, Performer, LG 56.62, P64LE19 and Pro 144. The first two hybrids belong to the old generation of hybrids (created 20 years ago), while last three hybrids belong to the new generations of hybrids (created 10 years ago). The hybrids of sunflower were cultivated in field experiments within the experimental farm of the Faculty of Agriculture, University of Agronomic Sciences and Veterinary Medicine of Bucharest.

For each hybrid of sunflower, determinations on quality pollen, and a correlation between pollen amount per flower and self-fertility were carried out. In addition, some morphological aspects of the flowers were tracked under magnifying glass.

Our results showed that crude protein has not recorded significant differences between hybrids, but we obtained differences between hybrids regarding the amount of ash and total sugar. Pollen yield has varied between 0,41 and 0,76 mg pollen/flower. To highlight the influence of genetic over years on the pollen of sunflower, our data obtained on hybrids pollen were compared with data on varieties pollen existed in literature.

Forewing Differences Between Honey Bees From Banat and Central Serbia

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Abstract:

The first written record of the varieties of honey bees in Serbia can be found in the work of Grozdanić (1926) who performed morphological comparisons of honey bee samples from Banat with *A. m. carnica*, *A. m. ligustica*, *A. m. cypria* and *A. m. syriaca* and suggested the separation of the bees from Banat into subspecies *A. m. banatica*. Due to the adaptable abilities of bees to specific habitat conditions in the Pannonian plain, it is assumed that different geographic ecotypes were created there.

Because of this the aim of our research was to analyse a part of morphological characters of honey bees from Banat and to compare them with honey bees from Central Serbia, in order to help distinguish and preserve indigenous honey bee varieties.

The samples were collected in three different locations in Banat and one location in Central Serbia. In this study, 14 morphometric characters were measured in accordance with the standard method. By means of a variance analysis very significant differences were determined between the examined bee groups. On the basis of LSD test we have determined that some honey bee groups from Banat differed very significantly ($P < 0.01$) from honey bees in Central Serbia regarding A4, D7 and E9 angles. Honey bees from Uljma (Southern Banat region) differed very significantly from all examined groups of honey bees as regards the size of O26 angle ($42.11 \pm 3.03^\circ$). Group of bees from Central Serbia had a very significantly larger width of forewing in relation to Banat honey bees. Investigation of morphometric characters on the forewing could contribute to a clearer separation of groups of bees inside domestic population.

Key words: honey bee, morphometry, forewing, ecotype, Serbia

Molecular Diagnosis of Main Viral Diseases in Regions of the Center and Southeast of Mexico.

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Abstract:

Diseases transmitted by viral pathogens that infect the honey bees have been reported in many countries, however, in Mexico there is a limited number of reports about main viral pathogens that affect the honey bees. This study was conducted for the diagnosis of main honey bee viruses: Sacbrood virus (SVB), Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), Black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV) and Kashmir bee virus (KBV), in samples of honey bees larvae of different apiaries from regions of the Center and Southeast of Mexico. Results obtained of the RT-PCR technique, demonstrated the presences of the viruses DWV, SBV, BQCV, and ABPV in honey bees larvae, from regions of the Central and Southeast of Mexico, however, no samples was tested positive for the viruses IAPV and KBV. Sequences of the viruses SBV, DWV, ABPV and BQCV showed more than 99% homology with sequences reported in GenBank, of the National Center for Biotechnology Information, NIH. The present study proves evidence that main honey bee viruses (DWV, SBV, BQCV and ABPV), are circulating in honey bee larvae of apiaries from regions of the Central and Southeast of Mexico. In Mexico the real impact of viral pathogens infection in honey bees is uncertain, is important monitoring the presence and spread of these viruses to know the impact of them in the health of honey bees colonies in Mexico. In conclusion, these results demonstrate the importance of developing and maintaining a program of surveillance and control of main viral pathogens in honey bees of Mexico.

Colony losses in South Africa 2014/2015 - the capensis problem

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Abstract:

Since 2009 Questionnaires regarding colony losses have been circulated amongst South African beekeepers, both hobbyists and commercial, but response has been disheartening. At the beginning of 2015 an epidemic outbreak of American foulbrood hit the Western Cape and although no official numbers have been released newspapers publish numbers in the range of 40% of colonies being destroyed by AFB. For some unknown reasons AFB outbreak correlated with no response from beekeepers the *A.m capensis* area. For this reason we only focus on losses of *A.m scutellata* in its native distribution range where average colony losses steadily increased from since 2009 (48%) and amounted to 55% for the 2014/2015 period. The losses experienced by beekeepers was perceived to be the 'capensis' problem (71%) while other perceived causes include fire (8%), insecticides (6%), hive pests (5%) and absconding only 2%.

The high number of losses due to the on-going 'capensis' problem is indeed worrisome the problem is still persisting after 25 years. The spread of this social parasite can well be controlled by beekeeper management practises especially when migrating for pollination, but the problem seems to be increasing rather than decreasing.

Commercial *Bombus impatiens* as reservoirs of emerging infectious diseases of colony collapse disorder in central Mexico

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Abstract:

The commercialization of bumble bee colonies as agricultural pollinators in North American greenhouses is primarily mediated by industrially produced *Bombus impatiens* colonies. However, *B. impatiens* is also host of various viruses that have been associated with colony collapse disorder in honey bees, as well as hosts to a number of bumble beespecific pathogens and parasites. In this study, we used qPCR to screen adult worker bumble bees collected from 120 different greenhouses in central Mexico. Fifty-four locations were positive for one or more pathogens (45 %). The most frequently detected pathogen was *Apicystis bombi*, which was present in 32 colonies. Of these 32 *A. bombi* positive colonies, 15 were co-infected with at least one other pathogen or parasite, such as *Locustacarus buchneri*, *Nosema bombi*, or the viral pathogens ABPV, CBPV, DWV, IAPV and KBV. Routine use of this type of screening technology together with policy changes to restrict pathogen infested commercial bumble bees should help improve the selection of healthy commercial colonies of *B. impatiens* and could lead to a higher efficiency in greenhouse pollination thus providing better environmental conservation of natural *Bombus* spp. by preventing spillover of emerging infectious diseases (EIDs).

Complete Genome Sequence of *Paenibacillus larvae* MEX14 isolated from honey bee larvae from Xochimilco quarter in Mexico City.

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Abstract:

Paenibacillus larvae strain MEX14 is a facultative anaerobic endospore-forming bacterium that infects *Apis mellifera* larvae. Strain Mex14 was isolated from domestic bees larvae collected at a backyard in Mexico City. The estimated genome size was determined as 4.18 Mb and harbors 4806 coding genes (CDSs).

Detection of Nosema Pathogens in the Apiaries of the Tyumen Region, Russia

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Abstract:

For the first time the nosema pathogen was noticed by Dongoff-Leuckart in 1857, who took them for fungal spores. In 1863 Leydik revealed their similarity in form with spores of pebriny of silkworm *Nosema bombycis*. Russian mycologist N.V. Sorokin in 1882 showed, that they are cause of bee colonies death. He found many broadly oval spores from dead bees. Their size was 6 μm long and 2-3 μm wide. In his book "The vegetation parasites of people and animals" he named the pathogen disease Saccharomyces apium Sorok. and described it.

E. Zander in 1909 has described *Nosema apis*. Later a new pathogen – *Nosema ceranae*, isolated from the *Apis cerana*, was described by Fries et al in 1996. Further studies showed *N. ceranae* is a globally dispersed pathogen of *A. mellifera* by Klee et al., 2007; Williams et al., 2009; Fries, 2010; Yoshiyama, Kimura, 2011 and others.

Microsporidian infections of *A. mellifera* are usual in Tyumen region and disease symptoms were observed in colonies during the last 40 years regularly. In the period 2005to2010 we studied samples of bees from 106 apiaries in our region. 76.5% samples were infected with *Nosema*. (Domatskaya et al., 2010). In 2012 we studied 175 samples from 25 apiaries, which of 20 apiaries were infected with these pathogens. *N. apis* was identified in 44 samples from 9 apiaries. In 42 samples from 9 apiaries we found *N. ceranae*, and in 4 samples from two apiaries both pathogens were registered. In 2013 we studied 238 samples from 27 apiaries, which of are 6 apiaries *N. ceranae* and two apiaries both pathogens were discovered. In 2014 we inspected 15 apiaries, which of 5 apiaries were identified *N. ceranae*, and we found *N. apis* and *N. ceranae* in one apiary only. Our data suggests that apiaries quantity are reduced which *Nosema apis* can be find in the Tyumen region.

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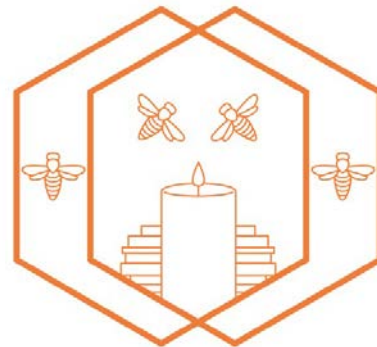
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