13th COLOSS Conference

Agricultural University of Athens, Athens, Greece
2nd-3rd November 2017

Ricola Foundation
Nature & Culture

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ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
AGRICULTURAL UNIVERSITY OF ATHENS

UNIVERSITÄT BERN

13th COLOSS Conference

TOPIC

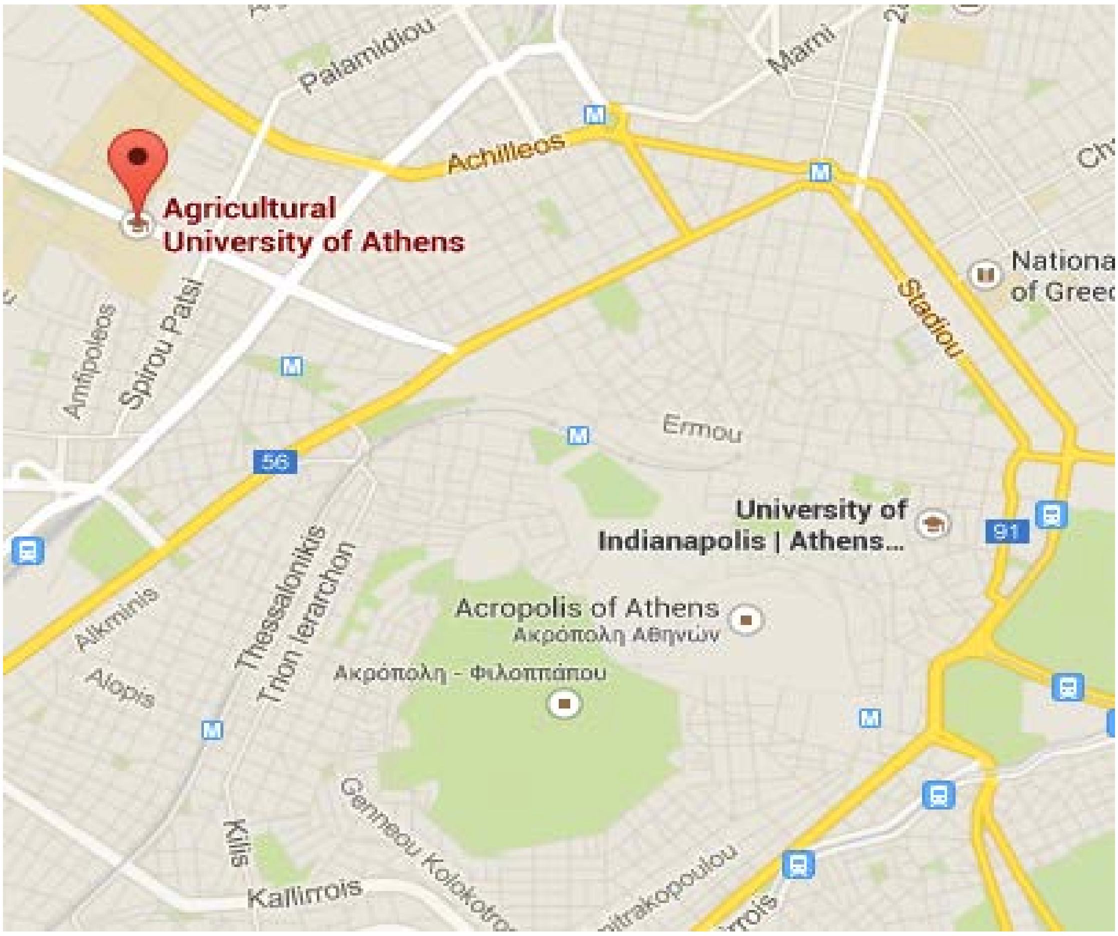
- International meeting of COLOSS to provide an update on the network's achievements and future directions, including meetings for Core Projects and Task Forces.
- Annual General Assembly Meeting

WHEN

1st November	Executive Committee Meeting in evening (open to EC members only)
2nd & 3rd November	COLOSS General Assembly and discussions (open to all COLOSS members)
3rd November	Executive Committee Meeting in evening (open to EC members only)

WHERE

Agricultural University of Athens, 75 Iera Odos, Athens, Greece



REGISTRATION FEE

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

POSTER SESSION

• Participants are invited to submit abstracts for a poster session prior to the social dinner. No submissions for oral presentations will be accepted.
• All abstracts must be accompanied by a poster
• Poster dimensions: no larger than **A0** (84.1x118.9 cm)
If the first author is a student, please sign your poster as “Student poster”. The best poster by a student will receive the prestigious COLOSS award.

FUNDING

- Registration fee will cover all coffee breaks, lunches, and the social dinner
- Due to limited financial support, participants will **NOT** be reimbursement for travel and accommodation.

Dear colleagues,

On behalf of the local organizing team, I would like to welcome you to the 13th COLOSS conference in Athens, Greece. It was my true pleasure to learn that so many people will participate even though this will be a stand-alone COLOSS conference this time.

I would like to sincerely thank all the people, who made this meeting possible. In particular, it would have been impossible without the exceptionally organized efforts of Maria Bouga and her local team.

Appreciation is also addressed to all contributors for submitting their abstracts, which I hope will stimulate rewarding discussions. Please be so kind and take in advance into consideration to plan our activity until the next COLOSS conference.

Financial support is kindly granted by the Eva Crane Trust, the Ricola Foundation *Nature and Culture*; Veto Pharma and the Vinetum Foundation.

I am looking forward to fruitful discussions with all of you, and hope you will enjoy this conference.

Yours sincerely,

Peter Neumann, President COLOSS Association

2017 COLOSS Conference - Tentative Schedule

Hosted by the Agricultural University of Athens, Athens, Greece

1 November 2017

Time	Session 1 – COLOSS Executive Committee Meeting 1 (for Executive Committee members only)
15:00-20:30	Meeting of the COLOSS Executive Committee

2 November 2017

Time	Session 2 – COLOSS General Assembly Meeting 1
08.30-09.00	**Sign-in & coffee**
09.00-09.15	Welcome by COLOSS President and Local Organizing Committee Chair
09.15-10:30	General Assembly Discussions
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 & 8 TFs; 8 mins. ea.)
12:30-14:00	**Lunch (covered) & poster set-up**
	Session 4 – Concurrent Discussion Groups 1
14:00-16:00	1. Monitoring, 2. Small Hive Beetle, 3. Vespa velutina
16:00-16:30	**Break**
	Session 5 – Concurrent Discussion Groups 2
16:30-18:30	1. CSI Pollen, 2. B-RAP, 3. Bee Breeding
	Session 6 – Posters & Social Dinner
18:30-20:00	**Poster session** with apéro
20:00-	**Social dinner**

3 November 2017

Time	Session 7 – Concurrent Discussion Groups 3
08:30-10:30	1. Varroa control, 2. APITOX, 3. Survivors
10:30-10:45	**Break**, with drinks & snacks
	Session 8 – Concurrent Discussion Groups 4
10:45-12:45	1. B-RAP, 2. Viruses, 3. Varroa Control
12:45-14:30	**Lunch**
	Session 9 – Concurrent Discussion Groups 5
14:30-16:30	1. APITOX, 2. Monitoring, 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
	Session 10 – Executive Committee Meeting 2 (for Executive Committee members only)
20:00-21:00	Debrief meeting of the COLOSS Executive Committee

ORGANIZER CONTACTS	
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Poster Presentations

Participants

NAME	LAST NAME	INSTITUTION
Alison	Gray	University of Strathclyde, Dept. of Mathematics and Statistics
Andrzej	Bober	National Veterinary Research Institute
Aneta	Ptaszyńska	Maria Curie-Sklodowska University
Aneta	Strachecka	University of Life Sciences in Lublin
Anna	Gajda	Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Laboratory of Bee Diseases
Anne	Dalmon	INRA
Aranzazu	Meana	Facultad de Veterinaria, Universidad Complutense de Madrid
Aslı	Özkırım	Hacettepe University Department of Biology
Aulo	Manino	University of Turin_DISAFA
Barbara	Locke	Swedish University of Agricultural Sciences
Beata	Panasiuk	Apiculture Division
Bjørn	Dahle	Norwegian Beekeepers Association
Bożena	Denisow	University of Life Sciences
Bram (ACM)	Cornelissen	Wageningen University and research
Bulet	Philippe	BioPark Archamps
Daniela	Laurino	Università degli Studi di Torino
Dariusz	Gerula	Research Institute of Horticulture, Apiculture Division in Puławy, Poland
Dirk	de Graaf	Ghent University
Elena	Facchini	Università degli Studi di Milano
Elias	Papadopoulos	Aristotle University of Thessaloniki Greece
Eva	Forsgren	Swedish University of Agricultural Sciences
Ewan	Campbell	University of Aberdeen
Fani	Hatjina	Division of Apiculture- Inst. of Animal Science- Hellenic Agricultural Organization "DEMETER"

Flemming	**Vejsnæs**	**Danish Beekeepers Association**
Franco	**Mutinelli**	**Istituto Zooprofilattico Sperimentale delle Venezie**
Gordana	**Glavan**	**University of Ljubljana, Biotechnical faculty, Department of biology**
Grazyna	**Topolska**	**Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Department of Pathology and Veterinary Diagnostics, Laboratory of Bee Deiseases**
Ivana	**Tlak Gajger**	**University of Zagreb Veterinary Faculty**
Jacek	**Jachuła**	**University of Life Sciences**
Janko	**Bozic**	**University of Ljubljana, Biotechnical Faculty**
Jevrosima	**Stevanovic**	**Faculty of Veterinary Medicine, University of Belgrade**
Jiří	**Danihlík**	**Department of Biochemistry, Faculty of Science, Palacký University Olomouc**
Joachim	**Rodrigues de Miranda**	**Swedish University of Agricultural Sciences**
Jorge	**Rivera Gomis**	**IZSLT**
Karina	**Antúnez**	**Instituto De Investigaciones Biológicas Clemente Estable**
Karl	**Crailsheim**	**University of Graz, Austria**
Laetitia	**Papoutsis**	**Agricultural University of Athens**
Linde	**Morawetz**	**Austrian Agency for Health and Food Safety**
Liydmyla	**Yazlovitska**	**Yuriy Fedkovych Chernivtsi University**
Lotta	**Fabricius Kristiansen**	**Apinordica**
Maja Ivana	**Smodiš Škerl**	**Agricultural institute of Slovenia**
Marco	**Pietropaoli**	**Istituto Zooprofilattico Sperimentale del Lazio e della Toscana**
Maria	**Bouga**	**Agricultural University of Athens**
Mariia	**Fedoriak**	**Yuriy Fedkovych Chernivtsi National University**
Marina	**Kosanovic**	**veterinary faculty**
Maritta	**Martikkala**	**Finnish Beekeepers Association**
Martin	**Kamler**	**Bee Research Institute Dol, Ltd.**
Martina	**Janků**	**Department of Biochemistry, Palacky University Olomouc**
Melanie	**Parejo**	**Switzerland**

Melissa	**Oddie**	**Institute of Bee Health**
Michail	**Kokkinis**	**Veterinary Centre of Thessaloniki**
Michel	**Bocquet**	**Michel Bocquet Consulting, personal entrerprise**
Milena	**Bajda**	**University of Life Sciences in Lublin**
Nanna	**Vidkjær**	**Aarhus University**
Noa	**Simon Delso**	**Centre Apicole de Recherche et d'Information (CARI)**
Nor	**Chejanovsky**	**Agricultural Research Organization, The Volcani Center**
Norman	**Carreck**	**International Bee Research Association**
Patricia	**Aldea**	**Universidad Mayor**
Per	**Kryger**	**Aarhus University, Department of Agroecology**
Peter	**Neumann**	**Institute of Bee Health, Vetsuisse Faculty, University of Bern**
Pike	**Brown**	**Landcare Research**
Preben	**Kristiansen**	**Swedish Beekeepers Association**
Quentin	**Rome**	**Muséum national d'Histoire naturelle**
Raffaele	**Dall'Olio**	**BeeSources**
Raquel	**Martin**	**Centro de Investigación Apícola y Agroambiental de Marchamalo (IRIAF)**
Remi	**Pade**	**Veto-pharma**
Robert	**Brodschneider**	**University of Graz**
Rosa Maria	**Licón**	**proApia**
Solenn	**Patalano**	**N/A**
Stefan	**Berg**	**Bavarian State Institute for Viniculture and Horticulture, Institute for Bee Research and Beekeeping**
Tamás	**Csáki**	**Hungarian Research Institute of Organic Agriculture**
Tjeerd	**Blacquière**	**Wageningen University & Research**
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Vincent	**Dietemann**	**Agroscope**
Yves	**Le Conte**	**INRA**
Panuwan	**Chantawannakul**	**Chiang Mai University**
Paschalis	**Harizanis**	**Agricultural University of Athens**
Nikolaos	**Panagidis**	**Agricultural University of Athens**
Evangelos	**Papas**	**Agricultural University of Athens**
Vaia	**Voukata**	**Agricultural University of Athens**

1. Influence of Honeybee Queens Storage on The Activity of Their Hemolymph Antioxidant System

Milena Bajda[1], Aneta Strachecka, Krzysztof Olszewski, Jacek Chobotow

[1]University of Life Sciences in Lublin, Poland.

Nowadays, commercial mass rearing of honeybee queens combined with their storage in cages or in mating hives (ca. 2500 bees) is a standard procedure, particularly in Central Europe, e.g. in Poland. The cage environment, however, is significantly different from the hive one. It may be assumed that the commercial intensification of queen rearing, particularly the cage storage of queens, leads to reduction in their quality, including their biochemical defense system, which comprises, i.a. the hemolymph antioxidant system. The aim of the research was to determine enzymatic antioxidant activities (SOD (superoxide dismutase) and CAT (catalase)) and TAC (total antioxidant capacity) levels in the hemolymph of 8-day-old virgin queens which had been kept in queen cages or in mating hives from their emergence. Two queen groups were created on the day of their emergence. In the first one, the queens (n=70) were individually placed into 70 queen cages, 10 worker bees with candy in each, and kept for 7 days there. Then, 10 pooled hemolymph samples, with the hemolymph of 7 queens in each, were taken. In the second group, the queens (n=70) were individually placed in 70 mating hives without the possibility of mating flights and kept there for 7 days. The hemolymph sampling protocol was the same as in the first group (10 pooled samples). The activities of SOD, CAT and the level of TAC were determined with a commercial kit in each sample. The enzymatic antioxidant activities and TAC levels were higher in the hemolymph of the queens kept in the mating hives than in those kept in the cages. As expected, the queens kept in the mating hives had more sufficient biochemical defense. Higher antioxidant system in the mating hives may be associated with a more diverse diet, better care of bees, defensive mechanism of neutralization of xenobiotics, and better protection against ROS (reactive oxygen species) and oxidative damage of proteins and DNA. Consequently, the higher activity of the antioxidant system may make for better biochemical defense, that in turn is related to less stress. It also affects the ability to detoxify metabolites and shows higher antipathogen activities compared to the caged queens.

2. Molecular characterization of *Nosema ceranae* present in Poland

Andrzej Bober[1], Dagmara Zdańska[1], Marta Skubida[1], Krystyna Pohorecka[1]

[1]National Veterinary Research Institute, Pulawy, Poland.

The aim of the study was to assess the phylogenetic diversity of Nosema ceranae strains and potential routes of introduction of the parasite to national apiaries. Polish isolates and isolates occurring in other countries were compared to obtain this aim. The research material comprised of samples of worker bees collected from individual colonies, originating from apiaries located in 16 provinces. To evaluate the phylogenetic relationships 3 samples with N. ceranae from different apiaries in each province were taken. In order to obtain DNA fragments (of the genes encoding polar tube proteins - PTP1, PTP2, PTP3) phylogenetic analysis was performed after a PCR using 4 primer pairs for each sample (for PTP1 gene 2 primer pairs were used). Analyses set up for the phylogenetic tree for PTP2 gene sequence and PTP3 gene sequence revealed the presence of one main group with the tendency to form subgroups for both the PTP2 gene and the PTP3 gene. Based on a comparison of genetic distances between the isolates we demonstrated very high similarity to the reference sequences for both fragments of analyzed genes.

3. BeeTyping™, a Biotyping-like mass spectrometry approach for bee health monitoring

Michel Bocquet[1]

[1]Michel Bocquet Consulting, personal enterprise, France.

Honeybee population decline is being attributed to stressors such as parasites (viruses, Varroa mite), pesticides, and environmental changes. Most researches focused on identifying the stressors' presence instead of their impact on honeybee colonies. BeeTyping™ aims at profiling the infected bees' immunoproteome, in order to deliver practical applications for bee health management. Hemolymph samples were collected from individuals from monitored colonies with a diagnosed infection, and from individuals artificially infected with a pathogen. Virus presence was confirmed by quantitative PCR. Protein content was analyzed and compared by MALDI-MS, directly or after reduction-alkylation of the hemolymph. Top-down analysis by LC-MS/MS was conducted to confirm protein identities. Hemolymph analyses tracked key peptides and proteins of the bee immunoproteome (apidaecin, hymenoptaecin…), and resulted in different molecular fingerprints in function of the bees' infectious conditions. These differences were confirmed by statistical comparison of MS profiles by principal component analysis. Virus-infected bee, with or without Varroa destructor co-infection, ended up in a cluster of their own inside the overall Varroa cluster. These first results strongly support the robustness of our monitoring approach in the case of co-infections, its potential as a plausible strategy to monitor honeybees' health, and a mean for a better understanding of the molecular immune response of this social insect, in both experimental and natural infections. Other infection models are currently being investigated, notably for microsporidia (Nosema) and entomobacteria.

4. Oxybee® (containing oxalic acid) in the treatment of varroosis in honey bees under field conditions in Germany

G. Braun[1], B. Lohr[1], N. Dany[2], C. Schneider[1], K. Hellmann[1]

[1]Klifovet AG, Munich, Germany. [2]Dany Bienenwohl GmbH, Munich, Germany.

A clinical field study in honey bees naturally infested with *Varroa destructor* was conducted to evaluate the efficacy and safety of the product Oxybee® in Germany from November 2012 to April 2013. Oxybee® is a veterinary medicinal product containing oxalic acid, for trickling application to control varroosis in honey bees. A total of 45 colonies were enrolled at 2 study sites, one in Southern and one in Northern Germany. Safety evaluation was based on: Bee mortality, colony and queen survival until the following spring, colony strength in the following spring, and area of open/sealed/drone brood in the following spring. The results showed that Oxybee® was highly efficacious and safe in the treatment of Varroosis in honey bees caused by *Varroa destructor* under field conditions in Germany. Oxybee® is one of the first Varroa medicine for honey bees to receive a positive opinion regarding a centralized authorization in Europe. It will be distributed starting end of 2017 by Véto-pharma.

5. Winter losses and reneweal during beekeeping season; outcomes from four year study in the Czech Republic and Austria

Robert Brodschneider[1], Martina Janků[2], Jan Brus[3], Karl Crailsheim[1], Jiří Danihlík[2]

[1]University of Graz, Graz, Austria. [2]Palacký University Olomouc, Department of Biochemistry, Faculty of Science, Olomouc, Czech Republic. [3]Palacký University Olomouc, Department of Geoinformatics, Olomouc, Czech Republic.

In Austria the monitoring of honey bee colony losses during winter is established since 2007/2008. In the Czech Republic, winter losses of honey bee colonies are investigated since 2013/14. In both countries, data are collected online, per e-mail or mail, and the surveys are advertised in beekeeping journals and at several beekeeper meetings. Winter losses between countries differ, but correspond by showing similar trends in high or low loss rates. Winter losses are influenced by many factors, e.g. Varroa treatment, altitude or robbing between colonies. Czechia and Austria have different strategies in Varroa treatment. Whereas Austrian beekeepers are mainly focused on treatment with organic substances, the synthetic treatment during summer time (Gabon strips) or fumigation during autumn time is approached by the majority of Czech beekeepers. The Czech Republic belongs to the group of countries with the highest colony density in Europe. Registration of beekeepers, their colonies and apiaries are mandatory in the Czech Republic. Therefore, an on-line map with colony density could be produced as a practical tool for beekeepers for finding a particular area with low colony density (http://colosscz.webnode.cz/hustota-zavceleni). Czech-Austrian cooperation is based on the COLOSS questionnaire for monitoring colony losses and supported by bilateral mobility program AKTION. The data collected during four year cooperation allow modelling changes in colony number in both countries. Interestingly, the number of colonies in Czechia and Austria increased based on our model, underlining the importance of summer renewal of honey bee colonies. This is in agreement with Czech central database of beekeepers and colonies.

6. Winter 2016 Honey Bee Colony Losses in New Zealand

Pike Brown[1], Linda Newstrom-Lloyd, Barry Foster, Paul Badger, John McLean

[1]Landcare Research, Wellington, New Zealand.

Estimating winter losses for managed honey bee (Apis mellifera) colonies is critical for understanding hive productivity and health. This study reports estimates of overwinter colony losses in New Zealand, which has seen exponential growth in the number of managed colonies in recent years. Over 35% of all beekeepers and 50% of all commercial beekeepers in the country responded to the internet-based 2016 New Zealand Colony Loss Survey, providing detailed information on over 275,000 colonies (over 40% of all registered colonies) that entered winter 2016. Using three different methods, we estimate overall winter losses to be below 10%. However, nearly 29% of beekeepers lost more than 15% of their colonies over winter 2016, and nearly 25% of beekeepers lost more than 20% of their colonies over winter 2016, indicating considerable skewness. These results are subject to strong regional variation, with the highest losses reported in areas with significant mānuka resources; similarly, non-commercial beekeepers report substantially higher losses than commercial beekeepers. Beekeepers who lost colonies over the winter of 2016 most frequently attributed the cause to colony death, queen problems, or wasps. However, varroa and competition for apiary sites were also identified as important areas of concern.

7. Identifying stressors and effectors of the honeybee immune response, through mass spectrometry, may represent a promising solution for bee health monitoring

Philippe Bulet [1]

[1]BioPark Archamps, Archamps, France.

In recent years, populations of western honeybees have declined worldwide. This decline is attributed to many stressors. Up to today, research and methodologies deployed against new challenges (such as use of pesticides, virus prevalence and other pathogens, climate and flora changes) have focused on evaluating and attempting to separately prevent and fight each factor. In addition, most of studies have focused on identifying and quantifying the presence of stress agents, instead of focusing on their impact on the colonies. The aim of our studies is to address these limitations by establishing robust, effective and sensitive technologies for profiling & deciphering bee immunoproteomes with regards to the host-pathogen interactions. The objective is to deliver practical applications for monitoring and enhance bee immunity for an integrated and adapted health management. The analyses of bee hemolymph, by hyphenated MALDI-MS and LC-ESI-MS/MS approaches for proteomic characterization of the immunoproteomes, resulted in visually different molecular profiles in function of the bees' infectious conditions (virus, Varroa mite, microsporidia Nosema). These differences were confirmed by statistical comparison of mass spectrometry profiles and discriminant analysis. We have demonstrated for example that virus-infected bees samples, with or without Varroa co-infection, ended up in a cluster of their own inside the overall Varroa cluster. This strongly supports the robustness of our monitoring approach in the case of co-infections, its potential as a plausible strategy to monitor honeybees' health, and for a better understanding of the molecular immune response of this social insect, in the context of experimental/natural infections.

8. On-farm research program for varroa control in organic beekeeping

Tamás Csáki[1], Dóra Drexler[1]

[1]Hungarian Research Institute of Organic Agriculture, Hungary.

Varroatosis as the current bane of the beekeepers is causing the biggest economic damage in the apicultural sector. Consistent control of varroatosis should be provided without harmful effects. In conventional operations varroa mites are usually treated by synthetic products that over time lose efficiency and leave toxic residues in the hive products. Adsorbent resin filtered honey imported from uncertain origin dominates the European bulk honey market. Therefore more and more beekeepers are trying to gain a viable position on the market with distinctive certification like the Organic. In the technology of organic beekeeping only natural materials are allowed to be used such as essential oils and organic acids that actually also have a renewed interest because of the because of the resistance to acaricides. Since 2013 within the beekeeping on-farm research program, the Hungarian Research Institute of Organic Agriculture is collaborating with 50 beekeepers throughout Hungary in comparative trials for testing the efficacy of different types of varroa control treatments and management. The trials are set up in market operations. Because of the high density of the hives in Hungary the reinfection is fast during the active season. The essential task of the program is to find an appropriate Varroa control method during brood presence. The current comparison is between the recently appeared extended release application method of the oxalic acid - glycerine solution to the vaporization application methods of lactic and oxalic acids.

9. Virus TaskForce (VTF)

Anne Dalmon, Marina Meixner, Per Kryger, Orlando Yañez, Nor Chejanovsky[1]

[1]Agricultural Research Organization, The Volcani Center, Israel.

Research on honey bee viruses is a quickly expanding frontier and there is an increasing need to address the appearance of new viruses, to establish standards to assess virus diversity and to gain better knowledge of the variability of viruses. These questions were brought together by Anne Dalmon (France), Marina Meixner (Germany), Per Kryger (Denmark), Orlando Yañez (Switzerland) and Nor Chejanovsky (Israel) to form the Virus TaskForce that met last April in Avignon (organized by AD and NC). The meeting was attended by 24 researchers from13 countries. Seven talks were the basis to discuss about virus variability, new viruses, biological assays, sample collection and transport. As a conclusion of the discussions several tasks were identified and defined: 1- To write together two reviews, one on regarding routes of infection and the other about virus distribution and prevalence in different countries/regions, making a database of what viruses are present in different countries. 2- To make a catalogue of old samples available in each lab (that will be studied for the presence of DWV B). 3- Design a sampling strategy to study virus variability and define an adequate sampling protocol including meaningful metadata. Then it was raised the need to list first the primers available for the different types of DWV, using different qPCR assays. Finally, it was agreed that the next meeting will take place in February 2018, in Warsaw, hosted by Anna Gajda. The meeting will be reported to the General Assembly of COLOSS.

10. Keep Calm - It's Only 365 Days Until Eurbee8

Dirk C. de Graaf[1]

[1]Ghent University, Belgium.

We are pleased to announce the 8th EurBee Congress of Apidology, to be held in Ghent, Belgium, September 18 – 20, 2018. This meeting follows the Eurbee congresses in Udine, Prague, Belfast, Ankara, Halle, Murcia, and Cluj Napoca. The EurBee congresses are held every two years and are a major international forum for discussion of the latest and most important results in bee research. The meetings bring together scientists from different fields and serve as a venue for exchanging ideas that emerge from basic and applied research. EurBee has become the premier event for researchers studying different aspects of wild and managed bees, and how they respond to environmental changes to address problems with species conservation, pollination services, beekeeping management and colony losses. The venue of Eurbee8 is the Campus Ledeganck of Ghent University, which is located near the historic centre of Ghent, a medieval city in the heart of Europe. More detailed information on deadlines for paper submission, registration and accommodation is available on the congress website: http://www.eurbee2018.org/

11. Fluctuation of pollen resources in ruderal plant communities. Data from SE Poland

Bożena Denisow[1]

[1]University of Life Sciences, Poland.

Pollen is necessary for bee development and survival, therefore the declines of bee pollinators could be partly related to changes in their host plant abundance and pollen quality. In Poland, blooming of ruderal plant species started in early spring and lasted until late summer (with the peak in mid-summer). The amount of pollen available differs considerably between the patches of vegetation, time periods within a growing season, and growing seasons. Inter-year disparities in total pollen yield available have been affected by natural processes of plant species expansion and displacement. Pollen yield of particular plant species ranged from 0.06 g/m2 (Crateagus monogyna) to 9.65 g•m-2 (Papaver rhoeas). Species considered as invasive produced high amount of pollen: Solidago gigantea 6.25 g•m-2 and Helianthus tuberosus 6.33 g•m-2 . The changes in pollen resources and pollen yield between plant patches (45.3 kg•ha–1 - 600 kg•ha–1) and temporal variation (0.5 - 8 kg•ha–1 – in spring; 41.6 – 83.1 kg•ha–1 in summer) were considerable. The protein content in pollen ranged from 16.1% (Prunus spinosa) to 58.2% (Echium vulgare). Mosaic structure of vegetation patches within the landscape seems to be necessary to fulfill spatial and temporal gaps in insect pollinators food resources. Propagation of invasive species (e.g. S. gigantea, H. tuberosus) in spite of their good pollen value, should be monitored.

12. Honey bee colony losses in Argentina during 2015-16.

Fabrice Requier[1], Nancy Garcia[2], Lucas A. Garibaldi[1]

[1]Instituto de Investigaciones en Recursos Naturales, Agroecología y Desarrollo Rural (IRNAD), Sede Andina, Universidad Nacional de Río Negro (UNRN) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mitre 630, CP 8400, San Carlos de Bariloche, Río Negro, Argentina, [2]Centro Pyme Adeneu, Agencia de desarrollo económico del Neuquén, Neuquén, Argentina.

The concerns for the ecological, social and agronomical consequences of honey bee decline have spurred several large-scale monitoring of honey bee colony losses around the world to evaluate potential drivers of decline. United States and Europe already have well developed such an approach, however, no large-scale monitoring of colony losses is yet developed in South America. We present preliminary results of such an initiative started in Argentina since 2015. We used the same core questions considered as "essential" in the COLOSS questionnaire with a Spanish translation and some adaptations to South American biogeographic conditions such adjustments of timing, names of diseases and botanical origin of honey. Given that the carrying out of citizen science programs in developing countries is challenging (i.e. limited access to information and communication networks for part of citizens), we performed two participatory methods: face-to-face interviews (an active method) and an online questionnaire (a passive one). Calls to respond to the online questionnaire were promoted through beekeeping journals and websites, and email appeals. The face-to-face interviews were performed by various beekeeping coordinators associated to the *National Beekeeping Consortium* – created for this purpose. In total, we received answers from 104 beekeepers, who collectively managed 30,546 colonies on this beekeeping season 2015-16, an estimated 1% of all managed colonies in Argentina. The magnitude of voluntary participation confirms the difficulty to gather a large amount of data through citizen science programs in South America compared to developed countries. The estimation of national annual colony losses was 15.6%, with 3.4% of losses during the summer and 14.5% during the winter. This rate of winter colony losses in Argentina is placed between the European estimation (12.0%, "overall winter loss rate") and the estimation in United States (22.3%, "total winter loss") for the same year. Beekeeper opinions suggested that the three main causes of their colony losses would come from sanitary issues (35% occurrence in answers), climate disorders (26.3%) and pesticide exposures (25.3%). Interestingly, the answers to the rate of colony losses during the summer were affected by the participatory method. Indeed, beekeepers that answered to online questionnaire lost 6.5% of colonies in average during the summer *versus* 2.9% for beekeepers that answered to face-to-face interviews. This result suggests that beekeepers suffering higher losses are more sensible to calls to respond to the online questionnaire. Such a methodological bias could lead to discussion about the potential over-estimation of (online) passive methods to colony losses monitoring. Perspectives are now to reinforce this colony losses monitoring in Argentina for the upcoming season 2016-17 and to expand this initiative more widely in South America.

13. Looking for Varroa's Achiles heel in Varroa chemosensing

Nurit Eliash[1, 2], Nitin K. Singh[1], Starlin Thangarajan[1], Noa Sela[1], Dena Leshkowitz[3], Yosi Kamer[1], Ilia Zaidman[1], Ada Rafaeli[4] & Victoria Soroker[1]

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The parasitic mite of honeybees, *Varroa destructor*, and its vectored viruses are today‘s main problem in beekeeping worldwide. The Varroa lifecycle is tightly linked to that of the bee and is apparently regulated by bee volatiles sensed via perforated sensillae located in the pit organ on the mite's foreleg. Intervention in the host chemosensing process could offer a target for new Varroa control methods. However, very little is known about the chemosensory mechanism in Chelicerata in general and Varroa in particular. To reveal the components of the mite chemosensory mechanism we implemented a transcriptomic analysis of forelegs, followed by a search for the presence of conserved domains of known chemosensory-proteins. This approach revealed transcripts of chemosensory related proteins belonging to several groups: Sensory neuron membrane protein (SNMPs), odorant binding proteins (OBP), Niemann-Pick disease protein, type C2 (NPC2), gustatory receptors (GRs), and ionotropic receptors (IRs). However, no insect odorant receptors (ORs) and odorant co-receptors (ORcos) were found. In addition, we identified a homolog of the most ancient IR co-receptor, IR25a in Varroa, bearing homology to other members of Acari. High expression of this transcript in the mite’s forelegs, while not detectable in the other pairs of legs, suggests a function for this IR25a-like in Varroa chemosensing. Studies on the function of this and other proteins are in progress.

14. Catch me if you can! Computed tomography analysis on a brood comb

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In the last years, honeybees *(Apis mellifera)* colonies losses have been recorded throughout Europe and the World. While a multitude of causative factors for this phenomenon is being debated, infestation with the invasive ectoparasitic mite *Varroa destructor* is now considered the most significant cause for important colony losses. The mites depend on honey bee brood for reproduction, and the reproductive cycles of host and parasite are tightly linked to each other. Within the isolated and protected environment of a capped cell, the mites and their offspring feed on the developing pupae. While the native host *A. cerana* has evolved a multitude of behavioral adaptations to limit the damage inflicted by the parasite, heavy mite infestation in colonies of *A. mellifera* causes severe damage, typically associated with secondary virus infections and a complex of symptoms known as varroosis, and will eventually lead to colony collapse. For research purposes it is important to optimize the methods to assess the mite infestation degree of a colony. Usually varroa brood infestation is assessed by opening a random sample of capped brood cells and measuring the percentage of infested cells. The purpose of the study was to assess the presence of varroa mite within brood cells with Computed Tomography. This imaging technique employs x-rays to produce cross-sectional images (slices) of a scanned object, allowing the visualization of its inner structures. A MDCT-scan was performed on a sealed brood comb from a colony in October 2016. Varroa was not detectable, but some pupae looked shorter than others. Therefore, the brood comb was inspected and whenever pupae looked shorter from MDCT-scan images, at least a single foundress mite was found within the same cell. Analyzing the length of varroa-free pupae compared to single foundress infested pupae, a statistically significant difference in length was found (t-Student, $P<0.01$). To our knowledge, this is the first time a honey bee brood comb has been analyzed with MDCT-scan technology. With proper implementation, MDCT-scan could become a fast and non-lethal approach to assess the infestation of brood combs from honey bee hives.

15. Colony losses in Ukraine – the third year of the survey

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This year we obtained 536 valid answers of the Ukrainian beekeepers from 23 of 25 administrative regions (including Donetsk and Luhansk regions). We collected the data after the winter 2016-2017 using Google form, by email, mail, by phone and by means of face to face questionnaire. We also published the COLOSS questionnaire in the Ukrainian Beekeeper Journal which allowed receiving some of the answers and acquainting more beekeepers with the survey. The overall winter loss rate of honey bee colonies during the winter of 2016/17 was 17.9 % (95 % CI 16.0-19.9 %). This is the highest overall winter loss rate in Ukraine during the last three years since we joined the COLOSS survey. It is 1.8 times higher than during the winter 2015/16 and 1.2 times higher than during the winter 2014/15. Still an overall loss rate in Ukraine is lower than the average result for the countries participating in the survey. Our respondents collectively wintered 20,846 colonies. The mortality rate reached 14.0% (95 % CI 12.3-15.9 %). Rate of loss of colonies due to queen problems was much lower than after the previous winter and made up 1.8 % (95 % CI 1.4-2.2 %). Colonies lost due to the natural disaster was comparatively high in Ukraine (2.1 %, 95 % CI 1.7-2.7 %). 15.4% (95 % CI 12.8-16.7 %) of colonies were weak after the winter, but with productive queens. The highest overall loss rate was in the mixed forest zone with insignificant differences among the physiographic zones of Ukraine. More than 80% of respondents in each physiographic zone of Ukraine treated their colonies against Varroa mite. The most common method was brood removal. Among chemical agents, Amitraz (in strips and fumigation), oxalic acid (trickling and sublimation) and Thymol were most often used. Individual respondents used some herbal remedies to treat their colonies: walnut leaves, thyme, tansy, horseradish fumigation.

16. Testing a CO_2 counter for assessment of phoretic varroa mites in bee colonies

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Flotation is the most recommended method for monitoring phoretic mites in bee colonies. However, this method requires killing sampled bees. The method which does not require killing bees is to shake out mites from bees powdered in icing sugar. The method that became popular recently is to shake out Varroa mites from adult bees that are anesthetized with CO2. The market already has suitable devices that are small in size and can be used in field conditions. The purpose of the study was to investigate the utility of the device with a CO2 dispenser for monitoring adult bee infestation by Varroa mites. The study was carried out on Sept 14th, 2016 in 12 colonies where no Varroa treatment had been undertaken before using CO_2 dispenser. For each colony, workers were collected to a 100 ml container, then weighed and transferred to the testing tool. There they were anesthetized and the mites were shaken. Subsequently, the bees were flotated to see whether the parasites remained on them. Average of 427 workers were found in the container. An average of 13.8 mites were reported after use of CO_2 counter while after additional flotation 10.4 more was observed. On average, during the tests, 62.5% of the mites had fallen, and the effectiveness of individual colonies ranged from 28.6 to 85.7%. This treatment is less effective than shaking out mites from the bees powdered in icing sugar. The effectiveness of this treatment was 77%.

17. A Comparison of Sublethal Effects of Nanosized Particles Zinc Oxide, Silver, Carbon Black, Titanium Oxide And Cerium (Iv) Oxide on Carnolian Honey Bee

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Deliberate application of nanopesticides and high annual industry production of various nanoparticles will result in inputs into the environment, by entering both in soil and in freshwaters. Honey bee is an important pollinator threatened by diverse environmental factors, potentially also by products of nanotechnologies. Different nanoparticles due to their different chemical properties could threaten honey bees in a different manner. Here we compare in vivo effects of sublethal chronic oral exposure of nanosized particles: zinc oxide (ZnO NPs), silver (Ag NPs), carbon black (CB NPs), titanium dioxide (TiO_2 NPs) and cerium (iv) oxide (CeO_2 NPs) on the honey bee survival, the activity of detoxification enzyme glutathione S-transferase (GST) and the activity of acetylcholinesterase (AChE). The activity of AChE is often used as an important biomarker of neurotoxicity after exposure to xenobiotics whereas GST was assessed as detoxification biomarker. In this study different concentrations of certain type of nanoparticles were tested in the same chronic experiment (9 or 10 days). The exposure of honeybees to ZnO and Ag NPs resulted in a decreased survival rate, whereas treatment with TiO_2, CeO_2 and CB NPs had no significant impact on honey bee mortality. The treatment with ZnO, Ag and CeO_2 NPs significantly elevated GST and altered AChE activities in honey bees. No alteration of the activity of AChE as well as GST was noticed in honeybees after exposure to TiO_2 and CB NPs indicating that TiO_2 and CB NPs at the tested exposure dose had no adverse effects on honeybees. Based on our findings we conclude that the potential use of TiO_2 and CB NPs in agriculture is currently safe for honeybees at the tested concentration level. On contrary, CeO_2, ZnO and Ag NPs have a neurotoxic potential and thus might contribute to honey bee survival.

18. Current and planned activities and reach of the monitoring group

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An ongoing goal of the monitoring group is to expand the representation of countries participating in the group, for a better overview of colony loss rates, and the group continues to be active in recruiting new contacts with potential to run their own national surveys. In 2017, 30 countries sent data from their monitoring survey to the international data co-ordinator for inclusion in the data analysis, a net increase of 1 country on last year. In fact data was received from Malta, Mexico and Serbia, as new countries to the monitoring group, and Belarus joined in once more, having taken part in 2015. Portugal has started monitoring but, owing to some local delays, their survey is in progress at the time of writing. We hope that it may be possible to include these data in a later analysis. Romania, Bulgaria, Bosnia-Herzegovina (a previous participant), Greece, Luxemburg and Armenia were also possibilities for monitoring, and remain so for next year. However as new countries join in, others are sometimes unable to continue. Some countries participating last time did not send data this year. Of those countries Turkey took part in 2016 after a break of a few years, but was unable to contribute in 2017. Lithuania had some difficulties with data return in 2016 and did not contribute at all this year. The Netherlands did not monitor this year, but has been a key contributor of data every year since the beginning of the monitoring group. Data quality remains an issue that hinders some of the analysis and limits the usefulness of some of the data collected. Special efforts were made to emphasise the importance of submitted data passing quality checks, through email communication, presentations, and also by including instructions to national co-ordinators as part of the codebook provided for data return. Despite this, some datasets did not comply with the coding rules, which delays analysis, and not all cases are useable owing to missing or inconsistent data. Disappointingly, for one country most of the data for some essential questions was missing. More support may be needed for new countries, but some more established contributors need to focus on these issues. Providing the codebook earlier and/or collaboration at the point of design of the local questionnaire and instructions to beekeepers may be necessary to reduce the problems encountered this year. A move towards more countries collecting data online may be helpful, for ease of access to the questionnaire and return of data by the beekeeper, as well as building in data consistency and quality checks. This will also allow new countries with widespread computer use to join in monitoring more easily with the support of those already doing online surveys. We hope to revisit the issues of the hot countries, in Africa and the Middle East, to give more support to our existing and former partners there and to recruit new ones. Connected to this is the participation this year of Mexico. Following the new initiative started in 2016 to submit an annual short paper on winter loss rates before more in-depth analysis, the second of this series of papers has just been submitted. This will be followed by a press release at the time of publication, expected by the end of 2017, as a change to the usual timing of this. A descriptive study of Varroa treatments in Europe is underway at the time of writing. Several other papers are planned. A priority is a review of winter loss rates to examine spatio-temporal patterns.

19. Summary of the first results of the International online survey on honey bee toxicity events [2014 - 2016]

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In 2014, the APIMONDIA WG ‘Adverse Effects of Agrochemicals and Bee medicines on bees’ launched an International online survey for honey bee toxicity events (https://docs.google.com/forms/d/1rg24GTQuKeh9Z93iIkTgdxGcbWUlehuTWkBDCF47aQ/viewform?c=0&w=1) Honeybee colonies around the world are facing perturbing damages by a number of parameters and among them toxicants as plant protection or veterinary medicinal products, as well as their possible synergy. This survey it was not designed to draw statistical conclusions, rather to map the situation and to give a global idea of what and where is happening. Its aim was not to replace any detailed initiative taken by other organisations or countries. Therefore we present here some descriptive figures from the registered events.

20. Spontaneous forage flora in agricultural landscape in SE Poland

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Loss of many blooming plants in natural ecosystems and the spread of large monocultures in agriculture cause environmental perturbations affecting bees. The data on nectar and pollen yielding plants is the base for selection of most efficient forage flora for *Apis mellifera* and for wild Apoidea. The evaluation of forage flora was performed in chosen rural municipalities in Lublin Upland, SE Poland. Richness and diversity of forage plant species were compared between road verges and field margins. For evaluation of pollinator food resources, node interrelation factor (NIF) was applied. Suggestions on bee pastures creation/supplementation have been incorporated. In agricultural landscape of SE Poland, the spontaneous forage flora richness and diversity differ among the types of man-made structures. Forage species richness and diversity was higher on field margins than on road verges. In both types of habitats forage species cover was generally low (<20%). The coverage of road verges and field margins were predominated by non-forage plant species (e.g. Bromus sp., *Dactylis glomerata*, *Equisetum arvense, Artemisia vulgaris*). Basing on NIF values comparison, it was concluded that pollinators food resources are distributed unevenly and floral patches 'nutritional capacity' differs considerably between particular areas within landscape. For that reason, pollinator food niches support seems unevitable. For the conditions of agricultural landscape of SE Poland, creation/supplementation of 6-9 forage flora patches of 0.025-0.3 ha each within an area of 100 ha is suggested.

21. Heat and Ozone in Beekeeping

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With a 30% average of colony over-winter mortality and reuse of hives and frames, the need for eco-responsible and reliable methods of honey bee pest control and of disinfection of used material has become more relevant than ever. A decontamination method is thus equally important. Honey bee colonies have for decades been chronically exposed to different acaricides. These have not provided full efficacy, have sometimes created parasitic resistance and also resulted in an increased build-up of residues from the different treatments. In addition, bees are exposed to plant protection products that often accumulate in beeswax. Residues may migrate into honey through diffusion and cause potential adverse synergistic effects to honey bees and humans. Beeswax is also used as a food additive and as a pharmaceutical and cosmetic ingredient, it is therefore important to have a residue-free product.

A biomechanical method is proposed here by the use of a device with a triple function:

1. **Deparasitation** Use of a device that injects heat of 42-44°C for up to three hours into a populated hive, prior removal of the queen, to drastically reduce the charge of *Varroa destructor* and potentially that of the small hive beetle (SHB) *Aethina tumida*. The same heat generator can be used to ensure elimination of all development stages of the wax moth *Galleria mellonella* before the storage of combs.

2. **Disinfection** With the addition of ozone (O_3), the same device could be used in unpopulated hives containing frames with empty wax cells (free of brood, honey and bee bread) in order to eliminate even the most resilient pathogen, *Paenibacillus larvae*, the agent of American foul brood (AFB).

3. **Decontamination** The above function serves also to reduce chemical residues from used combs. A supplier could also treat beeswax with O_3 before transforming it into foundation. Flakes of decapping combs after honey extraction are very thin and could easily be washed with ozonized water sufficient to dilute the honey, leach out water soluble particles, disinfect and decontaminate. Ozonized air could be blown in, should further treatment be required.

Rationale for the use of hyperthermia as *Varroa* control Heat against varroosis has been used since the 1970s, based on the observation that mites are more sensitive to temperature increases than bees. However, since then hyperthermia has received little research attention for the following reasons: most of the related experiments were made at a time when the decline of honey bee populations was not as severe as now; chemical products were cheap and simple to use; problems of residue accumulation in beeswax were negligible and *Varroa* resistance was not yet critical. Currently, there are on the market at least 12 heat devices that claim safety and *Varroa* control efficacy, However, not all of them have had proper scientific validation.

Co-infestation of bees with *Varroa* and SHB is becoming more common. These arthropods are more thermosensitive than bees: *Varroa* can tolerate up to 40°C (Harbo, 2000), the SHB up to 35°C (Meikle, 2011), the wax moth up to 35°C (Warren, 1962) compared with bees that tolerate up to 45-50°C (Elekonich, 2009). Hyperthermia to fight those pests may thus become relevant again, with humidity, CO_2 and time of exposure as important factors to consider. After all, the extreme thermotolerance could allow honey bees to use heat as a natural fighting mechanism against *Varroa* infestation (Hou, 2016), Asian bees to kill hornets (Ugajin, 2012) and Africanized bees to kill resident queens in colony takeovers (Vergara, 1993).

Rationale for the use O_3 as a sustainable sanitation technology Ozone is a powerful oxidant that has been used with increasing frequency as disinfection and decontamination methods in the food industry and in the medical field as it is more effective than chlorine and other disinfectants, while not leaving any harmful residue.

Ozone as a disinfectant Gaseous O_3 is a method that ensures full surface sterility. Ozone is also commonly used to sterilize water when it is bubbled into it. In turn, water enriched with O_3 can be used instead of traditional washing and disinfecting agents. In beekeeping, work from James (2011) demonstrated that O_3 could eliminate even spores of AFB from beekeeping equipment, and understandably all other pathogens and pests. Back then, destruction of AFB bacteria and spores required high concentrations (8560 mg O_3/m^3) and long exposure periods (3 d). However, it is not known if further studies of the application of O_3 have been performed in beekeeping lately. Since 2011, many improvements have occurred in O_3 disinfection methods, with Humidizone ™ as an example (Anonymous 2017). Disinfection conditions of bee nesting materials thus need to be reassessed with updated technology.

Ozone as a decontaminant Ozone was demonstrated to reduce levels of the neonicotinoid insecticides thiacloprid and imidacloprid within aqueous solutions (Černigoj et al, 2007). In beekeeping, James et al (2013) also showed that ozone drastically reduces the amount of coumaphos and tau-fluvalinate from beeswax. A treatment of heat and ozone can render contaminated and even heavily wax moth-infested frames acceptable again (James, 2011). Therefore, O_3 could be an innovative approach if routinely used to effectively disinfect beeswax and hives and also to decontaminate or at least reduce some of the beeswax residue contents.

An integrated approach via treatment of preferentially local or well adapted bees with organic acids and biotechnology such as brood elimination, queen caging, hyperthermia and a sanitization method with a small environmental footprint and may combine to result in the most sustainable solution for pest and contamination control. The use of synthetic, lipophilic acaricides in colonies should be minimized. It is important to consider that as soon as there is a choice between contaminating treatments and residue-free disinfection and decontamination methods, consumers will choose the latter.

NOTE: This project proposal requires scientific and financial assistance for its implementation.

22. "Varroawarndienst" - a beekeepers' Citizen Science project to support the control of Varroa mites

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The negative effects of the infestation with *Varroa destructor* is one of the major problems of modern beekeeping. Despite intensified training and regular information campaigns, Austrian beekeepers keep struggling with severe winter losses due to the Varroa mite. The "Varroawarndienst" (Varroa warning service) is a new approach, which aims to raise awareness of the Varroa problem and gives active advise to beekeepers to take action in situations of high Varroa infestation rates. In this Citizen Science project beekeepers are invited to share their data on Varroa infestation levels in their own colonies with all other beekeepers in Austria. They systematically sample natural mite fall (five times a year, each time for one week) and submit the collected data via a web-browser to a database where the data is analysed. In addition, the exact location of the apiary, from which the data was collected, is submitted. In return, they are provided with useful tools which evaluate the current state and development of Varroa infestation level of the beekeeper's own colonies. Each beekeeper visiting the platform gets an overview of the current situation of Varroa infestation in Austria. A classic traffic light design helps to catch the overall situation at one glance. Furthermore, visitors are provided with a detailed prediction of the expected development of the Varroa infestation level and of the situation in various regions of Austria. Information of high infestation levels in their regions will shift their attention towards early signs of Varroosis or reinfestation in their own colonies. A weather forecast is also implemented into the platform, which helps to find the ideal date or period for each particular type of Varroa treatment. Therefore, beekeepers can react in time and efficiently to the current situation and the probability that the bee colonies will survive the forthcoming winter is increased.

23. Multi-region loss rates of honey bee colonies during winter 2016/17 from the COLOSS survey in Italy

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We present loss rates of honey bee colonies during winter 2016/17 obtained with the COLOSS questionnaire in 12 regions of Italy. Unfortunately, not all the answers provided could be considered complete or valid. The 395 beekeepers providing valid loss data collectively wintered 13,392 colonies, and reported 916 (6.8%, 95% confidence interval (95% CI): 6.0-7.8%) colonies with unsolvable queen problems and 1,455 (10.9%, 95% CI: 9.6-12.3%) dead colonies after winter. Additionally colonies lost due to natural disaster amounted to 195 (1.5%, 95% CI: 1.1-1.9%). This results in an overall loss rate of 19.2% (95% CI: 17.5-20.9%) of honey bee colonies during winter 2016/17, with marked differences among regions. Trento province and Sicily region recorded 28.3% (95% CI: 18.9 – 40.2%) and 2.3% (95% CI: 1.1 – 4.8%) dead colonies after winter respectively; Trento province and Piedmont region reported 18.1% (95% CI: 11.4 – 27.5%) and 0.5% (95% CI: 0.0 – 18.0%) colonies with unsolvable queen problems respectively. The worst overall loss rate of 46.4% (95% CI: 34.7 – 58.6%) was reported in Trento province while the lowest one 10.7% (95% CI: 7.1 – 15.7%) in Emilia Romagna region. Overall, migratory beekeeping had no significant effect on the risk of winter loss. Data collected through COLOSS questionnaire seems interesting and could strongly contribute to the knowledge of colony losses phenomenon. A wider participation in the questionnaire is strongly recommended since the responders currently represent less than 1% of the total number of beekeepers in the country.

24. *Varroa destructor* mite population level influence on the strength and survivability of *Apis mellifera carnica* and *Apis mellifera caucasica* bees in Poland

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The research was carried out in the years of 2016 and 2017 in apiaries belonging to the Research Institute of Horticulture, Apiculture Division in Puławy, Poland. Tests were done in untreated bee colonies of 2 races: 14 *A. m. carnica* and 11 *A. m. caucasica*. In 3 week time intervals, between August and October 2016, natural mite fall after 48 hours, percentage of infested brood, number of frames with bees and number of frames with brood were checked. Then in colonies that survived winter, from March to May 2017, natural *Varroa* mites fall and colonies strength were checked again. *Varroa destructor* mite level in colonies of the two bee races was tested as a factor influencing colonies strength and survivability.

Differences were found in the *Varroa destructor* mite infestation level between two races of bees. Colonies with the highest mite insfestation did not survive the winter. No correlation was found between bee colonies infestation with varroa and strength of the colonies, however the tendency that the more brood in colonies late summer and autumn, the higher colonies losses occurred.

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25. Testing memory and adaptation in free-flying bees.

Solenn Patalano

Over the last decade, the collapsing of honeybee colonies has increased dramatically throughout the world. Numerous factors have emerged as responsible for the death of bees, such as the use of insecticides in agricultural environments, the emergence of new pathogens and parasites in hives or the poor genotypic adaptation of strains originating from uncoordinated breeding programs. Today, the current scientific consensus indicates a synergistic effect among all these factors as the main cause of the weakness of the colonies. It is therefore crucial to develop alternative approaches to anticipate the damage observed by a better and direct evaluation of the behavioural and cognitive bees' health within their natural environment.

Bees acquire effective cognitive skills such as memory and behavioural plasticity to ensure the health and productivity of the colony. Indeed, every day, forager bees must travel long distances, evaluate the flowers to pollinate, collect pollen and nectar and return to the colony. In order to repeat this task several times, bees must remember the location and type of the flower and sometimes adapt their path once the flowers have faded. In order to measure the cognitive factors that enable bees to orient themselves to collect nectar and pollen, a pilot study was carried out to test the memory and behavioural adaptation of several individuals from colonies of bees of various geographical and environmental origins. A typical Y- maze was used to train bees' memory and measure their adaptation capacity. This study shows how new methods can potentially establish a cognitive bee health index, which can anticipate the losses of colonies and bees' extinction.

26. Performances of two slow release formic acid products

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During summer 2017 (August), we carried out a field trial to evaluate the application of formic acid 60% administered with Nassenheider Professional® dispenser, compared to Varterminator® treatment to control V. destructor infestations. Concurrently, we assessed the toxicity of these above mentioned treatments on the honeybee colonies.
Nassenheider Professional® was used with 290 ml of formic acid 60% (APIFOR60) and the smaller U-wick size in order to have the slowest release of formic acid avoiding toxicity due to the high temperatures reached in summer in Central Italy.
Varterminator® is a veterinary medicine that consists in two gel tablets of 250 grams each containing 90 grams of formic acid to be applied for 10 days on the top of the frames in the brood chamber and substituted with two other tablets to leave in place for ten days more.
The field trials were undertaken in one apiary of 24 colonies divided into three homogeneous groups: 8 colonies were treated with formic acid 60% administered with Nassenheider Professional® dispenser for a period of 20 days (APIFOR60 GROUP); 8 colonies were treated with Varterminator® for a period of 20 days (VARTERMINATOR GROUP); 8 hives were left untreated to understand natural mite mortality (CONTROL GROUP).
After the 20-days treatments, we evaluated the number of mites killed inside the brood cells by counting mite fall for 12 days after the formic acid application. To evaluate residual mites, we counted mite fall over 21 days of queen caging and we performed a single dose of trickled oxalic acid solution (10:100:100) in absence of brood, prolonging the mite fall count for 7 days. The amount of adult honey bees, capped and uncapped brood cells after treatments was statistically similar between groups. Acaricide efficacy was: 61.3%±23.9 in APIFOR60 GROUP; 76.0%±16.7 in VARTERMINATOR GROUP and natural mite fall was equal to 37.0%±18.1.The number of dead queens after treatments was 1 per group (12.5%).
The residual amount of formic acid in the Nassenheider Professional® dispenser was 19,2±13,4 ml.The use of slow release formic acid products (Nassenheider Professional® with 290ml of formic acid 60% and the smaller U-wick size or Varterminator®)is an affordable strategy in temperate climate if we consider the reduced toxicity on honeybee colonies and the mean efficacy obtained in presence of brood.

27. Monitoring of *Vespa velutina*

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The Yellow-legged hornet, *Vespa velutina* nigrithorax, originates from eastern temperate to subtropical zones of Asia (Villemant et al., 2011); it was unintentionally introduced in France before 2004 (Haxaire et al 2006) via the importation of Chinese pottery for horticulture (Arca et al 2015). In France, the spread of *V. velutina* has been followed for twelve years using a monitoring protocol based on citizen warning and local networks: today, the hornet invasion extends in almost all French territory and reached the neighboring countries (Spain, Portugal, Italy, Germany, Belgium, Great Britain, Netherland) (http://frelonasiatique.mnhn.fr/home), progressing at a rate of about 60 km per year (Rome et al. 2013; Robinet et al. 2016). Also introduced in South Korea in the early 2000s, it arrived in Japan in 2015 (Kishi and Goka 2017). We described here the monitoring data validation protocol used to reliably confirm the locations where *V. velutina* is able to establish. We also show how these presence data registered in the INPN database (INPN 2003) are used to make predictions on hornet expansion (Villemant et al 2011; Barbet-Massin et al 2013)

28. New Frontiers in Varroa Control – Are HBAs an Effective Treatment Against Varroosis?

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In recent years, a tendency of increased use of organic products for varroa control is present. There are some preparations known and registered for organic repression on the base of plant extracts. Recently, products based on hop beta-acids (HBA) are offered as an alternative, since they are perceived as harmless, naturally occurring in the environment and consequently their maximum residue limits (MRLs) are set as not relevant. An important industry in Slovenian agriculture is also hop growing, which annually produces 2,500 t of hops and hop products, which ranks Slovenia in the 5th place in the world with 3% of world production. Consequently hop industry is always looking for alternative uses for hops outside the brewing industry. In addition to the role of the hops in beer as a raw material, which gives beer flavour and aroma, it is very important also the role of a preservative, because beer is one of the rare drinks with no addition of preservatives. In beer HBAs have a protective role with a proven antimicrobial effect. In our experiment we carried out practical testing of hops extracts in the form of toxicity tests on varroa infested bees at different concentrations and with different methods of application. We evaluated the effects of hop extracts on the cleaning behaviour of bees. Moreover, a pilot trial on colonies was carried out taking into account the environmental factors that may affect the condition of the colony. After conducting a pilot experiment, sensory evaluation of honey will be performed in order to determine the possible transition of hop components in honey, and possible impact to sensorial properties. The completion of this research will be a prototype product based on beta-acids on which stability tests in laboratory environment will be conducted. From existing gene banks of standard varieties and collections of new hop breeding lines we will search for suitable hop genotypes. We will determine the content of alpha- and beta-acids, and the quantity and composition of essential oils. Based on the results we will create a list of prospective genotypes suitable for cultivation for the purpose of extracting beta-acids. In commercially available hop extracts we will further determine their chemical composition of hop resins and essential oils, as a potential disruptive element.

29. Honeybee workers with higher reproductive potential are resistant to stress

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This study was funded by the National Science Centre (NCN) of Poland (grant 2014/15/B/NZ9/00425). Swarming is one of the most spectacular events in the life of a honeybee colony. After swarming, the rebels appear in the bee colony. Their appearance results from a temporary queen absence. Behavioural changes occurring during swarming are associated with the development of the larvae which have emerged from the eggs laid earlier by the previous queen. The rebels emerge from these larvae after pupation. Their ovaries are made up of a higher number of ovarian tubules. Their pharyngeal glands are reduced, and Dufourt glands enlarged, as compared with the normal workers. The same as the queen and in contrast to the sterile, normal workers, rebel workers are set to reproduce and do not participate in the rearing of successive bee generations. In order to fully comprehend the specific, physiological separateness of rebel workers, it is necessary to investigate age-related biochemical changes. The key substances responsible for bee resistance, vitality and longevity, particularly in conditions of high stress and senescence, are antioxidative enzymes. Therefore, the aim is to comprehend the physiological separateness of the rebel workers by studying senescence changes in their antioxidant system activities. In five unrelated original/source colonies, each kept in a two-box hive, rebel workers (*Apis mellifera* L.) were reared as described by Woyciechowski and Kuszewska (2012). One-day old rebel workers (RWs) were marked with a different colour from the normal workers (NWs) and introduced into mini-colonies. Haemolymph was collected on the 1st, 3rd, 6th, 12th, 18th, 24th and 30th day of worker age from RWs and NWs according to Strachecka et al.'s (2014) method. Following haemolymph collection, RWs and NWs were used for histological preparations of pharyngeal, mandibular and Dufour gland tissues and ovarian tissue to confirm particular subcaste status. Using commercial Sigma kits, the activities of the following enzymes were assayed in the haemolymph solutions: superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT) . SOD, CAT and GPx activities rose along with the age of the RWs and NWs, while GST activity was observed to increase only in the NWs. SOD, CAT and GST activities were lower in the RWs, whereas CAT activity was lower in the NWs. The tendencies observed in the RWs suggest physiological separateness of this subcaste in relation to NWs.

30. Determining the presence of bacteria *Paenibacillus larvae* in samples of beeswax comb foundations

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American foulbrood of the honeybee is an infectious contagious brood disease that is caused by the spore-forming bacteria *Paenibacillus larvae*. By regular annual examinations of honey and pollen stores, combs, and hive debris it is possible to determine the presence of spores of the infectious agent, which are the source of infection before any clinical signs could be noted. Also, examinations can be used as a confirmation for successful sanitation of this widespread and destructive disease. In modern intensive beekeeping, beeswax is a honeybee product that is constantly and regularly recycled by refinement into comb foundations. Since comb foundations are a necessary material to establish honeybee colonies, and are as well needed for honeybee colony management during the main pastures, it is extremely important they are not contaminated by spores of *P. larvae*. Because of increased number of clinical visible changes with suspicion on American foulbrood, intensive uncontrolled transportation of hives and increased positive results of lab testing during last few years, the aim of this study was to isolate and identify *P. larvae* from comb foundations. Samples were taken in specialized apiary stores or in wax production craft units in Croatia and five other countries. Internal laboratory protocol was modified from few previously published articles. Establishing a laboratory method would make it possible to routinely conduct early diagnostics of infectious agent of American foulbrood of the honeybee as a part of beeswax quality control test, and at the same time would control the production of comb foundations and would carry out preventative measures to prevent clinically visible disease in apiaries.

31. Spatial distribution of winter 2016/2017 colony losses in Poland vs. some climatic conditions

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In Poland we started the monitoring of honey bee colony losses after the winter of 2007/2008. Since 2009 the COLOSS questionnaire has been used for this purpose. All possible ways of disseminating questionnaires were utilized, that is: publishing in beekeeping journals, distribution during beekeepers' meetings and conferences, sending e-mails and letters to beekeepers. This method of implementing the survey resulted in a disproportion between beekeepers' participation in different regions. In the years 2014-2016 monitoring was based on stratified randomized sampling. Stratified randomized sampling turned out to be expensive and time consuming. In 2017 we used online LimeSurvey software (survey administered by Flemming Vejsnæs within Nordic Baltic Countries cooperation). The questionnaire and the link to the survey was published in beekeeping journals and sent to e-mail addresses provided by beekeepers during previous years' monitoring and to e-mail addresses of beekeeping associations. Up to June 30, 2017, we received 491 questionnaires with valid data on colony losses (311 collected in the Lime Survey and 180 received in e-mails and through the post). The overall colony loss rate of 491 beekeepers wintering 23193 honey bee colonies in autumn 2015 was 21.8%. Compared to the previous winter, in which the overall loss rate was 15.9% this is an extremely high loss rate. Losses due to queen problems and losses caused by natural disasters were included and reached 6.1% and 0.9% respectively. We found differences in colony loss rates between voivodeships ranging from 9.9% to 38.4%. The highest losses were observed in the Lubusz, Opole, Silesian and Pomeranian voivodeships, and the lowest in Swietokrzyskie, Kuyavian-Pomeranian and West Pomeranian. Analysis of the average losses (average losses are less affected than overall ones by the losses in the few huge apiaries) shows that in south western Poland the highest losses were observed in those areas where in September 2016 and March 2017 the temperature was highest, whilst in the northern part of Poland in those areas where in November 2016 precipitation was highest and temperature in March 2017 was lowest (also in comparison to the other regions). As in previous years beekeepers whose bees foraged on maize lost more colonies than beekeepers who claimed that their bees did not have access to such plantations (25.5% and 20.9% respectively).

32. The antioxidant system of honey bee under different temperature conditions during wintering

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Over the recent years, the number of honey bee colonies has been declining, especially after wintering. The resistance of bees to wintering is determined by a number of metabolic processes. One of the factors that negatively affects the health of bees, causing the death of entire colonies, is strong fluctuations in the temperature of the environment. Reactive oxygen species (ROS) are formed by all organisms in a number of pathways. Increased ROS levels lead to lipid peroxidation (LPO). As a result, thiobarbituric acid reactive substances (TBARS) can be used as a marker for LPO and oxidative stress in general. Antioxidative enzymes protect the bee's organism against damage by ROS. In particular, catalase (CAT) directly degrades hydrogen peroxide, and glutathione-S-transferase (GST) catalases the conjugation of reduced glutation to a large number of xenobiotics. The purpose of our work was to evaluate the activity of catalase, glutathione-S-transferase and the level of TBARS in worker bees of Apis mellifera under different temperature conditions of wintering. The experiment was conducted on a local population of honey bees on the apiary of the Chernivtsi National University (December-January). To investigate the effect of cold, bees were treated with low temperatures for 45 days. One experimental hive was placed at 2°C, while a control hive was kept at 20°C. Every two weeks, bees were taken out of the hives and frozen in liquid nitrogen. Biochemical parameters were determined in extracts of different body parts (head, thorax and abdomen). The activity of catalase was determined by the Aebi method and glutathione-S-transferase by the Pascal method. The level of TBARS was measured using thiobarbituric acid according to the Plaser method. In contrast to warmth-treated control bees, cold-treated bees showed a significant increase of TBA-active products in the muscles of the thorax at the beginning of the treatment. At the end of the experiment one and a half months later, this parameter was similar to control levels. At the same time, in the tissues of the head and the abdomen no influence of wintering temperature on the levels of TBARS was detected. Cold treatment initially led to increased catalase activity in the abdomen. This activation also decreased with time and reached control levels by the end of the experiment. In contrast, catalase activity was decreased in head tissues. No activity changes of this enzyme where detected in the thorax. The transfer of bees to the cold led to an increase in the activity of catalase in the abdominal tissues. In the future, this figure decreased and reached the level marked for thermal maintenance. At the same time, in the tissues of the head transfer to the cold caused a decrease in the activity of this enzyme. GST activity was not affected by temperature. Our results indicate that the redox state of honey bee cells depends on the wintering temperature.

Prevention of Honey Bee COlony LOSSes

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