Research Institute of Horticulture
Apiculture Division in Puławy, Poland

COLOSS

Research Network for Sustainable Bee Breeding

Spring 2020 Workshop Proceedings

Puławy, Poland, 20-21.02.2020

Research Network for Sustainable Bee Breeding
The COLOSS Task Force for Bee Breeding
Workshop Agenda

Thursday, 20. 02. 2020		
9:00	**Welcome and organisational matters** Marina Meixner, Cecilia Costa Update on members, activities, publications	
9:30	**Varroa reproduction and cell recapping in resistant populations** Stephen Martin	
10:10	**German SMR project** Ralph Büchler	
	Discussion	
11:00	**Coffee break**	
11:30	**Genetic selection of the honeybee (*Apis mellifera* L.) in a northern climate** Segolene Maucourt	
12:00	**Mass storage of honeybee queens during winter in Canada** Andree Rousseau	
12:30	**An update on the Smartbees subspecies SNP analysis** Per Kryger	
	Discussion	
13:00 - 14:00	**Lunch Break**	
15:00	Trip to Kazimierz all participants	EurBeST Core Group Meeting core group members
19:00	**Dinner**	
Friday, 21. 02. 2020		
9:00	**The EurBest Project – report and discussion** Ralph Büchler	
10:00	**The honey bee as a model organism** Aleksandra Łoś	
10:30	**The German Cryoconservation project –an overview** Marina Meixner	
11:00	**Coffee Break**	
11:30	Additional presentations, updates on ongoing projects, open discussion	
13:00	**Lunch Break**	
14:00	Additional presentations, updates on ongoing projects, open discussion	

GENETIC SELECTION OF THE HONEYBEE (*APIS MELLIFERA* L.) IN A NORTHERN CLIMATE

Segolene Maucourt, Pierre Giovenazzo, Claude Robert

Laval University, Quebec, Canada

Animal breeding, in combination with developments in agricultural technology, has made remarkable progress in increasing production of many domestic species. However, these important tools are poorly exploited in the honeybee (*Apis mellifera* L.) industry because of the complex genetic and reproductive features of the bee. In recent years, new mathematical approaches have allowed the application of statistical models in honey bee breeding programs and the use of breeding values to improve genetic selection. The aim of our project is to adapt statistical models currently used for breeding in the Dairy and Pig Industry in Quebec Canada for the genetic evaluation and performance monitoring of the honey bee. This project will: 1) identify honey bee performance traits with high heritability within the colonies of the Centre de recherche en sciences animales de Deschambault honey bee breeding program; 2) develop a breeding plan to improve the genetic potential and produce superior breeding stock and 3) measure the impact male selection on the breeding values of honey bee colonies. This novel approach will improve honey bee performance of traits relating to yield stability and sustainability of our northern climate beekeeping industry.

ACCUMULATION OF LITHIUM CHLORIDE IN HONEY BEE TISSUE AND HIVE PRODUCTS OF AND ITS EFFECT ON THE COLONY

Jernej Bubnič, Uroš Kur, Janez Prešern

Agricultural Iinstitute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia

The mite *Varroa destructor* is an obligatory ectoparasite of the honey bee (*Apis mellifera*) and is one of the major threats to apiculture worldwide. Not only because of the direct effect on the honey bees, but also because they have a role as a vector for many bee viruses. There are some chemicals authorized by veterinary authorities to treat varroosis, the disease caused by mites, but two main problems of authorized medicines are resistance of mites or variable efficacy. Effect of lithium chloride (LiCl) on mites was discovered in 2018. Since then many beekeepers use it for treating their bees despite it is not authorized and “metabolism” of LiCl in honey bee colony is not known. In our study we investigated the effect of added LiCl in honey bee feed on concentrations of LiCl in adult worker bee tissue, larvae, hive products and on survival of adult worker bees.

First experiment was performed on caged bees in three different groups. First group was fed manually once with 10.6 µg of 25 mM LiCl in sucrose solution, the second group had ad libitum and the third group was control group fed only with sucrose solution. We monitored the mortality rate in cages. Samples of heads, thoraxes and abdomens from each group were collected for detection of LiCl. Second experiment was performed in

honey bee colonies that were fed with 1l of 25 mM LiCl in sucrose solution, three days in a row. Samples of 8 day old larvae, honey and pollen were collected and mortality of adult workers was monitored. LiCl detection was done by ICP-MS mass spectrometry.

We found out, that after manual feeding with 10.6µg of LiCl, up to 266 µg/kg of lithium accumulates in worker bee abdomens and that it has a negative effect on bee survival over a period of seven days. Average mortality rate was 71.67% in ad libitum fed group, 36.19% manually fed group and 17.86% in control group. In second experiment the highest concentrations of LiCl in larvae were three days post treatment, up to 46 µg/kg. the highest concentrations of LiCl in honey were again three days post treatment and were up to 1343 µg /kg. concentrations in pollen were up to 47 µg/kg. Concentrations of LiCl in control group were always below the limit of detection (0.5mg/kg). Increased adult bee mortality was observed in treated colonies.

Based on our results, we can conclude that treating colonies with LiCl poses serious threat to safety of hive products and health of honey bees. For future use of LiCl as active ingredient against varroa mite further tests are needed to develop application method that would reduce risk for food safety and honey bee health.

BEEWILD, A MOBILE APP TO A MOBILE APP TO RECORD AND MONITOR UNMANAGED COLONIES OF *APIS MELLIFERA* IN EUROPE

Paolo Fontana, Daniele Andreis , Stefano Corradini, Valerio Mazzoni

Fondazione Edmund Mach, Trento, Italy

The purpose of the beeWild mobile phone application is to create a register of the status of unmanaged honey bee colonies through citizens' reports. The application, which will initially be limited to Europe only, is created for the Android and iOS platforms. The application is aimed at both citizens and professionals (researchers and beekeepers). The reports of the presence and / or state of activity of the colonies can be made anonymously or as a report or as an authenticated subject (custodian). The App, created and owned by the Edmund Mach Foundation, will be managed, by formal agreement, by FEM, COLOSS TF Survivors and World Biodiversity Association, which together will validate the reports and will be able to use the data for scientific purposes and joined papers or projects.

MASS STORAGE OF HONEYBEE QUEENS DURING WINTER IN CANADA

Andree Rousseau (1), Emile Houle (1), Mireille Levesque (2), Pierre Giovenazzo (2)

(1) Centre de recherche en sciences animales de Deschambault, Canada

(2) Universite Laval, Quebec, Canada

The Canadian beekeeping industry requires an important number of honeybee queens in the spring of each year to replace winter mortality of colonies, for nucleus production and colony division. Canadian queen breeding industry cannot meet spring demand of queens because of limiting weathers conditions. Consequently, our industry is highly dependent on queen imports (from California USA mainly) at the beginning of the season. The goal of our project is to maintain locally produced mated queens live and fertile from September to April. To accomplish this, various queen banking/storage methods will be tested (temperatures below or above cluster formation and queen density in banks). Efficacy of tested methods will be evaluated by measuring queen survival, sperm viability within queen's spermatheca and the post banking performance of queens introduced in colonies the following season. Hopefully, results from this project will allow beekeepers to have access to locally raised queens early spring and thus reduce their dependency toward queen imports.

THERMAL DISPERSION OF DIFFERENT HONEY BEE HIVE MODELS AND THEIR CONSEQUENCES

Raffaele Dall'Olio

BeeSources

Is it possible to fully understand honey bee biology while mostly studying the animal through farmed conditions? Several factors related to different honey bee managment can trigger different responses in honey bee behaviour, being the nest and nesting site choice the main one. How this might also impact colony health? The evidences recently published by T. Schiffer will be discussed.

LIMITATIONS TO DARWINIAN BLACK BOX HONEY BEE SELECTION

Tjeerd Blacquiere (1), Willem Boot (2), Johan Calis (2), Pam van Stratum (2), Delphine Panziera (3)

(1) Wageningen University & Research

(2) Inbuzz vof

(3) Martin Luther University Halle-Wittenberg & German Center for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig

In 2019 we published an outline for Darwinian selection within a beekeeping environment for survival of western honey bees with un-treated *Varroa destructor* (Biol Invasions 21, 2519-28 DOI 10.1007/s10530-019-02001-0). The approach was based on more than 10 years of experience with 3 populations of honey bees in the Netherlands (NL). Basics: the colony (phenotype) incl. queens, workers, drones, but also parasites (*V. destructor*), microbiota (incl. viruses), is the selection level; selection for colony fitness only; no treatment of varroa; within population mating (remote mating areas). Benefits of the approach would be acquired natural resistance to Varroa, including local adaptation of populations by the avoidance of excessive travel and trade with bees. We assumed that having worked in NL, this approach would work on other places. To test this, we started 3 new programs in NL, Germany (G) (both 2018) and Belgium (B, 2019).

For NL and G, we are in the critical 2nd year, and in both populations the mating success of the queens under selection was lower than in the control group which received a Varroa treatment twice a year (G mating success: 100% control, 68% selection; NL mating success: 85% control, 68% selection. In Belgium all are still 'control' group (last treated in July 2019): 75% success. Survival in December 2019 of control colonies that had successfully mated in July, was 100% at all locations, but in selection colonies it was 87% in NL, and 0% in G, showing a complete extinction of the local population after just one year and a few months without treatment!

Several elements could explain this fast extinction: too narrow genetic base? Environmental factors? Differences in loads of viruses? Higher virus susceptibility? Ongoing analysis will give an insight on the original genetical variation of this population. In the meantime, we hope for success in our NL and B populations.

This research is funded by Bayer Bee Care, foundation Dioraphte and an anonymous foundation.

Keywords: *Apis mellifera*; Natural selection; Resistance; Tolerance; Sustainable apiculture, *Varroa destructor*

ARE HONEY BEES MODEL ORGANISMS FOR RESEARCH?

Aleksandra Loś (1), Małgorzata Bieńkowska (2)

(1) Polish Academy of Sciences, Institute of Nature Conservation

(2) Research Institute of Horticulture, Apiculture Division, Puławy, Poland

Insects perfectly fit the flagship principle of animal research - 3R: to reduce (the number of animals), to replace (animals with alternative models) and to refine (methods). Bees have the most important advantages of a model organism: they cause minimal ethical controversy, they have a small and fully known genome, and they permit the use of many experimental techniques. Bees have a fully functional DNMT toolkit. Therefore, they are used as models in biomedical/genetic research, e.g. in research on the development of cancer or in the diagnostics of mental and neuroleptic diseases in humans. The reversion of aging processes in bees offers hope for progress in gerontology research. The cellular mechanisms of learning and memory coding, as well as the indicators of biochemical immunity parameters, are similar or analogous to those in humans, so bees may become useful in monitoring changes in behavior and metabolism. Bees are very well suited for studies on the dose of the substance applied to determine the lethal dose or the effect of a formula on life expectancy. Honeybees have proven to be an effective tool for studying the effects of a long-term consumption of stimulants, as well as for observing behavioral changes and developing addictions at the individual and social levels, as well as for investigating the effects of continuously delivering the same dose of a substance. The genomic and physiological flexibility of bees in dividing tasks among workers in a colony makes it possible to create a Single Cohort Colony (SCC) in which peers compared perform different tasks. Moreover, behavioral methods (e.g. Proboscis Extension Reflex - PER, Sting Extension Reflex - SER, free flying target discrimination tasks or the cap pushing response) make it possible to analyse changes occurring in honeybee brains during learning and remembering. Algorithms of actions are created based on the behavior of a colony or individual, e.g. Artificial Bee Colony Algorithm (ABCA). Honeybees are also model organisms for profiling the so-called intelligence of a swarm or collective intelligence. Additionally, they serve as models for guidance systems and aviation technologies. Bees have inspired important projects in robotics, such as B-droid, Robobee and The Green Brain Project. It has also been confirmed that the apian sense of smell can be used to detect explosive devices, such as TNT, or drugs (including heroin, cocaine, amphetamines and cannabis). This inconspicuous little insect can revolutionize the world of science and contribute to the solution of many scientific problems as a versatile model (Los, A., Bienkowska, M., & Strachecka, A. (2019). Honey bee (*Apis mellifera*) as an alternative model invertebrate organism. Medycyna Weterynaryjna, 75(2), 93-106).

SCIENTIFIC CONSTRAINTS

Peter Neumann

Institute of Bee Health, University of Bern, Switzerland

Reliable identification of both wild and managed honeybee populations surviving Varroa destructor by means of natural selection constitutes a challenging task. It appears as if a combination of social and natural sciences is the way forward. This talk will provide an overview of both the challenges we are facing and suggestions for possible solutions.

OXALIC ACID FOR SUMMER VARROA TREATMENT IN BEE COLONIES

Paweł Węgrzynowicz, Małgorzata Bieńkowska, Dariusz Gerula, Beata Panasiuk, Tomasz Białek, Ewa Skwarek

Research Institute of Horticulture, Apiculture Division in Puławy, Poland

The aim of the experiment was to check the effectiveness of oxalic acid in summer *Varroa destructor* treatment. We studied 20 bee colonies kept in Dadant hives and equipped with hygienic bottoms. The strength of the colonies expressed in the number of bees and the number of cells with brood (Libefeld method) was assessed three times during the season (29.06,11.09.09.09.2017). Colonies were randomly divided into three groups: two experimental groups with 7 colonies and a control group with 6 colonies.

The experimental group queens were placed in the "Chmara" excluders for 24 days period (starting from 07.07.2017), while the queens in control colonies laid eggs throughout the whole period of experiment. Natural Varroa mite fall was checked every few days in all bee colonies. Three weeks later (28.07.2017), the queens were released from the excluders- brood absence- and anti-varroa treatments with oxalic acid began:

Group 1- 2.5 % oxalic acid, dose of 5ml / comb

Group 2- 4.2% oxalic acid, dose of 5ml / comb

Group 3- control: 2 Biowar 500 strips / colony.

Thirteen days after acid application (08.08.2017) both all experimental colonies (Groups 1 and 2) were checked for mite fall and 2 Biowar 500 strips were applied in. Dead mites were counted every few days, until 08.10.2017.

Total number of mites dropped during the whole experiment varied significantly between colonies, and ranged from 52 to 6594, average 2271 mites / colony. The differences between the average number of mites after treatments applied and for the whole experiment in particular groups were not significant.

The daily natural mite fall monitored until the use of oxalic acid or Biowar 500 ranged from 0 to 22.7 between the colonies (average 2.58 mites per day). In Group 1, where 2.5% oxalic acid was applied, average 412 varroa mites dropped on the bottom boards, while in Group 2, where 4.2% oxalic acid was applied, average of 1033 mites dropped, that was respectively 26.4 and 38.7% of the total number of *Varroa desturctor* females fallen during the entire treatment period. Total number of mites dropped during the whole experiment varied significantly between colonies, and ranged from 52 to 6594 (av. 2271 mites / colony). The differences between the average number of mites after treatments applied and for the whole experiment in particular groups were not significant. A significant relationship was stated between the natural *V.destructor* females fall and the mites that died after oxalic acid application ($r = 0.91$, $p \leq 0.05$) (as number of mites). A significant relationship was also noted between the number of parasites fallen after Biowar 500 application ($r = 0.45$), and the total number of *Varroa desturctor* mites during the whole experiment period ($r = 0.68$).

EFFECT OF TIMING OF QUEEN CAGING ON HONEY PRODUCTION

Marin Kovačić (1), Zlatko Puškadija(1), Leonidas Charistos(2), Fani Hatjina(2), Marco Pietropaoli(3), Giovanni Formato(3), Jerzy Wilde(4), Aleksandar Uzunov(5), Ralph Büchler(6)

(1) J.J. Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek, V. Preloga 1, 31000, Osijek, Croatia
(2) Hellenic Agricultural Organization "DEMETER", Institute of Animal Sciences, Division of Apiculture, Nea Moudania 63 200, Greece
(3) Instituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Via Appia Nuova 1411, 00178 Rome, Italy
(4) Department of Apiculture, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, Słoneczna 48, 10-957 Olsztyn, Poland
(5) Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food, Skopje 1000, Macedonia
(6) LLH, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany

Ectoparasitic mite *Varroa destructor* is a major cause of winter honeybee colony losses and is major apicultural research topic. Among many approaches in fighting *V. destructor*, recently brood interruption came into focus. A number of researches has shown that different brood interruption methods during brood rearing period can effectively reduce the infestation of colonies with mites before development of wintering bees. However, there is no much knowledge of the impact of brood interruption on honey production. Therefore, the aim of this research was to investigate does the timing of confiding the queen in the cage has influence on nectar intake during that period.

The research was performed in Croatia, Greece, Italy and Poland. At each research site 3 groups of 8 colonies were engaged. Briefly, in two group of colonies queens were caged in Var-Control Cage Mozzato (API-MO.BRU) for 28 days: one group (QC1) was caged 28 and second one (QC2) 14 days before expected honey harvest of the main summer flow. Additional group of colonies with free laying queens was used as control (C) and was treated against mites using standards local treatment. Colonies were weighted at the day of caging QC1 group, QC2 group and before honey harvest. Colony strength was measured at the day of queen caging, queen realise and end of experiment by number of combs with bees and brood. Infestation of adult bees with varroa mites was realised with soapy water wash or powder sugar shake method. Treatment of colonies was performed with oxalic acid on the day when queens were realised from the cage.

The mean net weight gains of the colonies did not differ between groups and ranged from 6,36 kg in group C to 7,09 kg in group QC2. As for countries, there was no significant differences, and net colony weight gain ranged from 5,72 kg in Italy to 7,48 in Poland. Until the end of the experiment (56 days after the queen caging), the colonies from QC groups lost significantly more bees comparing to control group (35% and 20% of bee population reduction respectively). Finally, the adult bee infestation with varroa mites was more reduced using the QC methods comparing to control group.

Under average season for honey production recorded on the last year may influenced the results of this research. Also, it would be interesting to see is there difference in honey production between QC methods and trapping the queen on a single comb where she will be free to produce brood. Further research is needed to provide more meaningful answers.

PARTICIPANTS LIST

Andonov Sreten	Macedonia	sreten_andonov@yahoo.com
Basso Benjamin	France	benjamin.basso@inrae.fr
Bieńkowska Małgorzata	Poland	malgorzata.bienkowska@inhort.pl
Bubnic Jernej	Slovenia	jernej.bubnic@gmail.com
Büechler Ralph	Germany	ralph.buechler@llh.hessen.de
Carreck Norman	UK	norman.carreck@btinternet.com
Charistos Leonidas	Greece	leoharistos@instmelissocomias.gr
Costa Cecilia	Italy	cecilia.costa@crea.gov.it
Cliceri Danny	Italy	danny.cliceri@gmail.com
Dainat Benjamin	Switzerland	benjamin.dainat@agroscope.admin.ch
Dall'Olio Raffaele	Italy	raffaele.dallolio@gmail.com
Dimitrov Lazo	Macedonia	L.Dimitrov@tera-consulting.com
Di Prisco Gennaro	Italy	gennaro.diprisco@crea.gov.it
Drazic Marica Maja	Croatia	marica.drazic@mps.hr
Elen Dylan	UK	d.elen@bangor.ac.uk
Filipi Janja	Croatia	jfilipi@unizd.hr
Fontana Paolo	Italy	paolo_api.fontana@fmach.it
Gajda Anna	Poland	anna.gajda83@gmail.com
Gasic Bozidar	Serbia	mikig@nodeurope.eu
Gerula Dariusz	Poland	dariusz.gerula@inhort.pl
Giovenazzo Pierre	Canada	pierre.giovenazzo@bio.ulaval.ca
Guichard Matthieu	Switzerland	matthieu.guichard@agroscope.admin.ch
Hatjina Fani	Greece	fhatjina@gmail.com
Kezic Nikola	Croatia	nkezic@agr.hr
Kovacic Marin	Croatia	komarin@fazos.hr
Kryger Per	Denmark	per.kryger@agro.au.dk
Licon Luna Rosa Maria	France	romaliconluna@live.com
Leleń Monika	Poland	lelenmonika@gmail.com
Łoś Aleksandra	Poland	los-aleksandra@o2.pl
Martin Stephen	UK	s.j.martin@salford.ac.uk
Maucourt Segolene	Canada	segolene.maucourt.1@ulaval.ca
Mazur Ewa	Poland	ewa_mazur@sggw.pl
Meixner Marina	Germany	marina.meixner@llh.hessen.de
Mondet Fanny	France	fanny.mondet@inra.fr
Moro Arrigo	Switzerland	arrigo.moro@vetsuisse.unibe.ch

Panasiuk Beata	Poland	beata.panasiuk@inhort.pl
Presern Janez	Slovenia	janez.presern@kis.si
Puskadija Zlatko	Croatia	zlatko.puskadija@fazos.hr
Raczyńska Aleksandra	Poland	info@vet-animal.pl
Raczyński Marcin	Poland	m.raczynski@vet-animal.pl
Rasic Sladjan	Serbia	rasic.sladjan@gmail.com
Richmond Deborah	Portugal	hello@deborahrichmond.co.uk
Rogenstein Steve	Germany	ambeessadors@gmail.com
Rousseau Andree	Canada	andree.rousseau@crsad.qc.ca
Soroker Victoria	Israel	sorokerv@agri.gov.il
Uzunov Aleksandar	Macedonia	uzunov@fznh.ukim.edu.mk
Valentini Adelaide	Italy	adelaide.valentini@gmail.com
Wilde Jerzy	Poland	jerzy.wilde@uwm.edu.pl
Węgrzynowicz Paweł	Poland	pawel.wegrzynowicz@inhort.pl

Minutes of the RNSBB Workshop, Pulawy, Poland, 2020

The COLOSS task force Research Network for Sustainable Bee Breeding held its annual Workshop in Pulawy, Poland, on 20-21 February 2020.

The task force shares the opinion that native honey bee populations represent an important resource for breeding gentle and productive bees adapted to environmental challenges. The aim of the task force is to improve honey bee welfare by developing and disseminating comprehensive breeding strategies that include colony vitality and the conservation of locally adapted populations. The group's working approach includes cooperation, exchange of ideas and mutual support.

During the Workshop in Pulawy several colleagues presented their research, and the outcomes, implications and perspectives were discussed.

Steve Martin presented his recent publication "*Varroa destructor* reproduction and cell re-capping in mite-resistant *Apis mellifera* populations" which he described as the culmination of 35 years research work. He is now confident that cell opening on behalf of the bees is a universal resistance mechanism that *Apis mellifera* populations enact wherever natural selection has a chance to take place (i.e. in untreated populations).

Ralph Buechler presented a study project commissioned by the EU Agriculture Directorate "EURBEST" aimed at supporting the development of breeding for varroa resistance, in which many RNSBB members are involved. Ralph also presented a project underway in Germany with the aim to detect and reproduce colonies with high recapping and SMR traits. An important part of the project is beekeeper training and involvement.

Marina Meixner presented an experiment that took place on the island of Malta, where the autochthonous *A. m. ruttneri* was compared to the commercially used *A. m. ligustica*. The comparison provides useful evidence of adaptation benefits of the local population, which will be useful to incentivate breeding of this subspecies on behalf of Maltese beekeepers. Marina also presented the German Cryoconservation project, conducted in collaboration with the Hohen Neundorf Institute, as a useful tool to contribute to conservation of honey bee diversity activities.

Andree Rousseau showed results from experiments aimed at improving queen banking over winter, with the aim of making locally bred queens available for beekeepers in the spring, as they currently resort to importation from abroad.

Per Kryger showed the final results of the SMARTBEES conservation working group, in which thousands of honey bees were sequenced and which had the practical outcome of a diagnostic tool (a SNP array) which can be used to classify a sample of bees into a subspecies category with a certain degree of probability.

Segolene Maucourt gave an update on her PhD project of Genetic selection of the honeybee (*Apis mellifera* L.) in a northern climate, showing the found heritabilities and success in selection.

All presentations triggered lively discussion and provided new research stimulus for the group.