Research Institute of Horticulture
Apiculture Division in Puławy, Poland

COLOSS

Varroa control

Spring 2020 Workshop Proceedings

Puławy, Poland, 18.02.2020

17.02.2020 welkome evening at “Tawerna” restaurant, 6 Sierpnia 43 street

(just by Trzy Korony Hotel) starting around 19.00 for those who want to attend

The COLOSS Task Force for VARROA CONTROL

Workshop Agenda

18.02.2020 Tuesday	
9.00	**Registration, welcome and administrative issues**
9.30 - 11.30	**Working groups** (WG1-WG6) -Presentations by each WG/ report of status and future perspectives
11.30 - 12.00	Coffee break
12.00 - 14.00	**Presentations of experiments and current work by members**
14.00 - 15.00	Lunch break
15.00 - 16:30	**Varroa book discussion - presentations of the chapters outlines**
16.30 - 17.00	Coffee break
17.00 - 18.30	**Further discussion and plans for future**
19… SOCIAL DINNER at TAWERNA	

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

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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)

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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

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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.

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MINUTES OF VARROA CONTROL TASK FORCE MEETING, PULAWY, 18.02.2020.

The Varroa control task force meeting was held in Pulawy on 18th of February 2020, coordinated by Marin Kovačić, Fani Hatjina and Victoria Soroker following election at annual Coloss meeting in Montreal.

The meeting was divided into four parts: presentation of current status of each of the 6 existing working groups, presentations of the group members, update of the Varroa book and discussion of future direction.

1. **9:30 – 11:30 presentation by each WG/report of status and future perspectives**

WG 1 – Varroa Infestation Assessment – report by Marco Pietropaoli via Skype

Members are working on publication entitled „Evaluation of on-field methods to assess Varroa mite infestation on honey bees“ and hopefully it will be finished in few months. Beside this, working group is currently not working actively. Marco proposed a few future activities:

- to compare the different tools and to test new technologies in Varroa mite infestation monitoring (i.e. beescaning.com, apisfero.org, bienengesundheit.at)
- make trials in order to develop “varroa population dynamics curves” in different areas (also considering different management practices/treatments).
- to compare data from the tools with the bottom boards varroa counts in some “efficacy trial”

WG 2 - Brood interruption – report by Ralph Büchler

The common Brood interruption publication is submitted to JAR (on 12.02.2020). The core group had a few day meeting in Mavrovo (North Macedonia) hosted by Alex, where the tasks and obligations were shared. The final manuscript was accepted by all participating members (except Marry Coffey from who Ralph didn’t get any answer) before the submition. Several members were active during last summer with new brood interruption study which goal was to investigate effect of queen caging on honey production and colony strength. The results of the study were presented in the next section by Marin.

WG 3 – CSI Varroa – report by Fani Hatjina

Protocol has been translated in different languages and it has not been changed. The announcement (invitation) in COLOSS website/ protocol/ updates will be done. The first part of invitation refers to possible coordinators (explanations of what to do). The second part of the invitation explains what coordinators will need to translate and refers to what the CS has to do, as well as how to do (detailed protocol). Future perspective is to continue with this working group, to update the announcement regularly, to communicate with the National coordinators more regularly and to start an online notification system – Lime survey. Dylan and Paolo showed interest to join the data collection. Ralph suggested to make some publication of results soon and to set a deadline. There is also a suggestion to join WG1 and 3.

WG 4 - Formic acid management – report by Benjamin Dainat

Basically, working group is finished. There is an effort to make a publication, however there is no much progress since Coloss meeting in Montreal. Following the first analyses, results were difficult to interpret and it was thus decided to give the dataset to a student in mathematics for new analyses. Previously missed problems in the dataset were identified (e.g. wrong time for treatment start, missing data ...) and improvements were done: e.g. a more formal method to determine if reinvasions occurred, exact treatment starting time identified. Dataset was corrected and basic analyses were repeated with corrected dataset: results of correlations between climatic parameters and treatment efficacy changed compared to first analyses. In depth analyses now need to be repeated before the manuscript is adapted.

WG 5 - Assessment of new control methods by Maja Smodiš Škerl (via skype)

Maja is on maternity leave and Jorge is working his PhD on pigs. Maja told that members of the group have to agree should we continue with group or not. If the group will stay, new leaders should be proposed. The members of the group performed trial to evaluate the performances of Varromed®, which ended in winter 2018-2019. Data from the participants was received and the publication assessing Varromed® performances is being prepared.

WG 6 – Communication – report by Victoria Soroker

The main activity of this group is the preparation of Varroa book. The book preparation is finally in progress. Discussion and chapter overview took place in the separate afternoon section.

After the working groups' overview, the general discussion followed. The main question that was raised is "what is the future of the task force"? At the moment, with so many working groups, we are missing the clear goal where we are going. The members were asked to suggest new research areas that would improve our knowledge in Varroa. Raffaele noted that we need more innovation and suggested that we do not have to test the new medicines against Varroa as this way we are doing favors to the producers of medicines. The general discussion among the participants of meeting led to the conclusion that we should not test the new medicines that show up on the market. Instead, there was suggestion from Per Kryger to test the efficacy of different registered medicines existing on the EU market with the purpose to give recommendations to beekeepers. One of the strength of the TF is that we are capable of doing large scale experiment that cover whole Europe (i.e. finished Brood interruption study). Ralph showed delight that three task forces (Varroa control, Survivors and RNSBB) are having meeting at the same time and that these groups are closely linked with the basic goal to better understand biology of Varroa mite. Paolo Fontana showed interest in the research of drone population dynamics in colonies and its effect on Varroa population development.

2. 12:00-14:00 Presentations of experiments and current work by members

There were a number of presentation of current work by members: Ralph presented results of the brood interruption study that is submitted to JAR. Marin presented results in front of the working group about the effect of queen caging on honey production and colony strength. The invitation was sent to members to contact Marin who is interested in repeating the research this season. Victoria presented the results of queen caging with oxalic acid treatments via trickling and sublimation in Israel. The presentations by Janez and Fani showed their results in using new methods in Varroa treatment: lithium and glycerox strips.

3. 15:00 – 17:00 Varroa book discussion – presentation of the chapter outlines

Norman presented the book status. All of the book chapters leading authors except for Joachim De Miranda and Flemming Vejsnæs presented their chapters' details, the list of authors and the chapter outlines. Some of the authors presented via Skype. Overall there is a considerable progress in the preparation of all the chapters.

As this book is meant mainly for beekeepers and extension specialists, the authors were advised to keep their writing accordingly and to avoid scientific terms as much as possible as well as detailed explanations of basic scientific issues. Moreover, as this book is regarded as a product of the entire task force effort the authors were encouraged to invite additional contributors while the audience was encouraged to participate in writing by direct contact with the leading authors. It was also suggested that prior to publishing the book, each of the chapters will be reviewed by a panel including the members of BEERAP task force. The names of all the reviewers will be listed in the BOOK.

In the effort to homogenize the structure of the book, within a few weeks Norman will provide detailed guidelines to the authors.

4. 17:30 - 18:45 Future perspectives

During the final discussion it was agreed that there is no reason to keep the division of the task force to the working groups. Instead, the tasks undertaken by the group members will be named "activities" of the TF.

In the end, the main activities that could be highlighted at the moment as main group current and future activities are:

- Monitoring Varroa infestation dynamics CSI-Varroa (Fani Hatjina)
- Evaluation of the significance of drone population to Varroa infestation (Protocol by Paolo Fontana and Ralph Büchler)

- Effect of colony distribution and density on reinfestation of colonies with Varroa mite (Tjeerd Blacquière is asked to prepare protocol)
- Brood interruption impact on the colony productivity (Marin Kovačić)
- Dissemination via Varroa book preparation

In the discussion that followed, Fani Hatjina suggested to look for the possibility for founding for the group activities. In particularly she suggested to apply for Marie Currie or Prima projects. Vicky was interested to compare Varroa resistance to acaricides. Tjeerd suggested that it would be interesting to repeat Smith and Seeley (2015) reinfestation experiment on the European scale. Benjamin emphasized the effect of climate change on colony brood and varroa development. Stephen Martin suggested to try to catch feral colonies that survive and try to examine the reasons why they survive. Zlatko Puškadija highlighted that it would be interesting to research other effects of brood interruption on the colony (i.e. viruses and other bee diseases).

Next Varroa TF meeting will be held during the annual Coloss meeting (before) the Eurbee conference in Belgrade. In that meeting the timing and location of winter/spring meeting will be decided. We are open to receive suggestions from the group members.