Hellenic Institute of Apiculture
HELLENIC AGRICULTURAL ORGANIZATION 'DEMETER'

Proceedings of the COLOSS.2 Training School

"Queen Quality and Breeding"

Organized by

Hellenic Institute of Apiculture, Hell. Agr. Org. 'DEMETER'

Thessaloniki, 2-4 March 2014

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Agenda

Saturday, 1st March 2014

Time	Activity	Location
19:00-	Gathering in the hotel, welcome drink	Capsis Hotel
20:00-	Dinner	Capsis Hotel

Sunday, 2nd March 2014

Time	Activity	Location
9:00-9:30	Registration	Capsis Hotel
9:30- 12:00	Meeting- discussions on methods related to queen and drone quality/ fertility. Speakers: (alphabetical order) Bieńkowska Malgorzata Costa Cecilia Giovenazzo Pierre Gregorc Aleš Hassan Rushdy Adel Hatjina Fani Demonstration- queen anatomy- sperm counting (?)	Capsis Hotel
12:30-13:00	Lunch	Capsis Hotel
13:00-15:00	Visit the Arcaeological Museum of Thessaloniki- free entrance http://www.amth.gr/images/filladia_multilanguage/pdf/en.pdf	
16:00-18:30	Meeting –Continue discussions and presentations/reports on experimental results Speakers: Kezic Nikola Tofilski Adam Uzunov Aleksandar Wilde Jerzy Williams Geoffry ……	Capsis Hotel
19:00-	Quick walk in the city/ Dinner	Ladadika area

Monday, 3rd March 2014

Time	Activity	Location
9:00- 13:00	Meeting- presentations by: Esmaeil Amiri Yasser Ibrahim - discussions for future activities within COLOSS, as part of RNSBB task force group	Capsis Hotel
13:00-13:30	Lunch	Capsis Hotel
13:30-17:00	Meeting –continue	Capsis Hotel
17:30-	Bus to the old town, walk in castle- Dinner- Walk back!	Castle area

Tuesday, 4th March 2014

Time	Activity	Location
9:00- 13:00	Meeting- devoted to RNSBB members and some specific issues relating to development of already initiated activities	Capsis Hotel
13:00-14:00	Lunch	Capsis Hotel
	Free time/ departure - or visit the institute of Apiculture in Nea Moudania DINNER	???

List of participants

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Multiple use of queenless colonies in mass honey bee queens' rearing

Malgorzata Bienkowska, Dariusz Gerula, Beata Panasiuk, Pawel Wegrzynowicz

Abstract:

Rearing the bee queens can be performed in the presence of the queen in a nurse colony however a higher effectiveness can be reached in queenless colonies and in the absence of emergency queen cells. In an extensive queen rearing when queen cells are incubated in a nurse colony, the colony can be used one or two times. In a continuous rearing, when the nurse colony is used repeatedly, it should be strong, healthy and with brood of different age. These conditions enable rise about 180 queen cells during one month of rearing. The aim of the observation conducted in the breeding apiary in Apiculture Division in Pulawy was to verify the efficiency of rearing queens with multiple usages of queenless colonies. Bee queens were reared in 61 queenless colonies in "Dadant" and "Wielkopolski" type hives with 3 brood and 2 storage (honey and pollen) combs. Rearing frame with 1-day-old grafted larvae was placed in the center of the colony. When capped queen cells were moved into incubators, the next series of grafted larvae was placed into rearing colonies. Each nurse colony was provided with the brood combs taken from support colonies every 10 days of rearing period. The efficiency of rearing queens was assessed as:

-% of incubated queen cells in relation to grafted larvae

-% of emerged queens in relation to grafted larvae

During three years of observation a total of 30 210 larvae were grafted into 61 nurse colonies in a total of 574 of rearing series (repetitions). Each rearing series (grafted frame) contained on average 55 larvae (from 45 to 110). Altogether 18 372 (61,8%) larvae were accepted and queen cells incubated.

There were no significant differences between percentages of accepted larvae in May, June and July of 3 years of queens' rearing. Significantly lower percentage of isolated queen cells was observed in August. Percentage of accepted larvae (incubated queen cells) in successive series, regardless the month of rearing, did not differ. Altogether in three years of observations in the highest number of nurse colonies (22-26) five series of honey bee queens were performed, then six to ten series were performed in 13 to 21 nurse colonies and even more than ten series in 1 to 13 nurse colonies. Percentage of accepted larvae did not differ in the first ten series of queens' rearing. Only in the

next series it was lower and came to 53.8%. Similar findings were done for the number of emerged queens.
Altogether of 358 queens were inseminated. The average body weight at emergence was 199,5 mg. To the insemination they lost about 30 mg of their initial weight reaching on average 170 mg. Among examined queens, 79.1% cleared oviducts, 18.7% had some semen residue and 2.2% were dead. The higher percentage of queens with some semen in oviducts was observed among light ones. Significant correlations were found between the weight at emergence and at insemination day and other factors: volume of spermatheca and number of spermatozoa

Weight of honey bee queens and its effect on the quality of instrumentally inseminated queens

Malgorzata Bienkowska, Beata Panasiuk, Dariusz Gerula, Pawel Wegrzynowicz

Abstract:
One of the quality criteria of honey bee queens is their weight at emergence. It depends on the age of larvae used for queens' rearing, season, strength and condition of rearing colony. The younger larvae used for queen rearing the heavier queens emerge. The aim of the research was to verify the influence of weight of queens at emergence and at insemination day on oviducts condition and a number of spermatozoa in spermatheca. The research was carried out in the Department of Bee Breeding, Apiculture Division in Pulawy, Poland. Carniolan bee queens were reared from 1-day-old larvae. They were inseminated at the age of 7 days with single dose of 8µl semen. Queens were kept in Zander cages before insemination in queenless colonies. After the insemination queens with 25 attendant workers were put into mailing cages and moved into queenless colonies. Queens were weight at emergence and at insemination day. The dead queens were counted 48 hrs after insemination. Surviving ones were killed and dissected to examine their oviducts for residue of semen. The volume of spermatheca was measured and the number of spermatozoa in spermatheca was counted.

Assessment of success of isolated mating of *Apis mellifera siciliana*

Cecilia Costa, Raffaele Dall'Olio
Consiglio per la Ricerca e la sperimentazione in Agricoltura
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Abstract:
A project for the reintroduction of the Sicilian black bee *Apis mellifera siciliana* is ongoing. One of the milestones of the project is to establish protected mating stations on mainland island. While working towards this, conservation of genetic lines on small islands is essential. Due to the small existing population, the big effort required in transporting the colonies and limited foraging resources on the islands, we would like to assess success of mating on the islands in comparison to mainland mating. Hence, the training school on queen quality will thus help us acquire the necessary skills to achieve this.

Testing a sublethal dose of imidacloprid and a formic acid varroa treatment on drone development and fertility

Anrée Rousseau[1], Radu-Ionut Apreutese[2], Marie-Odile Benoit-Biancamano[2] and Pierre Giovenazzo[1]
[1]Université Laval; [2]Université de Montréal

Abstract:
Twenty honey bee colonies from our honey bee research farm (Centre de recherche en sciences animales de Deschambault, Québec, Canada) were distributed randomly in four experimental groups : group 1 control, no treatment and a sucrose 1 :1 feeding solution; group 2 Varroa treatment (MAQS) and a sucrose 1 :1 feeding solution; group 3 imidacloprid 10 ppb in a sucrose 1 :1 feeding solution; group 4 Varroa treatment MAQS and imidacloprid 10 ppb in a sucrose 1 :1 feeding solution.
Treatments started July 11 2013 and lasted two weeks. Drone larvae were sampled at age 6, 12 and 18 days for histomorphological study of their reproductive system. Mature drones were sampled at age 15 days

for sperm study (sperm count, sperm live/dead viability test). Although previous studies put forth a consistent morpho-functional influence of pesticides treatments on the male reproductive system (Johnson et al., 2013) we failed to confirm those findings by light microscopic investigations. Furthermore we did not mesure any influence of various treatments on mature sperm.

Testing queens from commercial queen rearing apiaries

Aleš Gregorc
Agricultural Institute of Slovenia

Abstract:
The quality of the queen produced can be predicated by a number of variables that a colony often cannot control. During queen rearing, several conditions can be controlled and thus rearing procedures and optimum queen quality, which is of great importance, can be achieved. Altogether 888 queens were collected from queen breeding stations during the rearing seasons 2006, 2008 and 2010. Twelve queens from each apiary were sampled from the same selected queen mother colony and mated using typical technology for each apiary. Nine queens were dissected and prepared for morphological analyses and three queens from each sampling group were prepared for viral analyses on acute bee paralysis virus (ABPV), black queen cell virus (BQCV), deformed wing virus (DWV) and sacbrood virus (SBV). The ovaries and spermatheca were dissected, the volume of spermatheca was calculated and number of spermatozoa was counted using Bürker's haemocytometer. Ovary tissue sections on microscopic slides were then stained using Mayer's haematoxylin and Eosin and ovarioles were counted. The sampled queens' midguts were microscopically examined and *Nosema* spp. spores identified. Queens were examined on the presence of four viruses. The highest average queen weight of 209,49±9,82 mg was detected in 2008 and ovary weight of 78,67±11,86 mg in 2010. Average numbers of spermatozoa in queens ranged from 3.30 x 10^6 in 2006 to 5,23 x 10^6 in 2010. *N. ceranae* spores were found in queens sampled in 2008 and 2010, but not in queens sampled in 2006. Tested viruses were found in uneven incidences during testing periods. Results of the survey will help queen breeders and specialists in extension service in improving rearing technologies and mating conditions in queen rearing apiaries. Standard characteristics can

help in monitoring the queen parameters and monitoring the rearing practice.

Flow cytometry analysis of drone sperm

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Abstract:
Flow cytometry is fast, objective and reproducible method for analysing large amounts of cells. Apart from analysing size and structural complexity, its greatest advantage is in ability to detect fluorescences from various dyes for each cell or object in the sample. With such dyes as propidium iodide (PI) that binds strongly to DNA and permeates only dead cells it is possible to discern live versus dead cell populations in the sample. This is also possible to measure with time factor incorporated to measure cell viability or resistance to certain agents. With additional dyes amount of information can be easily increased. Requirements are of course that sample, cells, be in the fluid medium separated from one another and sufficiently diluted. Cells such as sperm that are naturally separated from each other are thus ideal target for flow cytometry analysis. Another advantage of flow cytometry in bee research is that it requires very small amounts of material to perform analysis. Already there have been described successful analyses of sperm of various species with PI and SYBR14 dye using flow cytometry. In our experiment we used cheap and reliable PI die to detect amount of dead cells in drone sperm. The analysis showed two peaks of fluorescence, one higher of dead cells with PI and one lower with live cells. This quick analysis is good for orientation but dye for live cells such as SYBR14 would be preferable to discern population of live cells from artefacts and thus increase precision of measurement.

Effect of Neonicotinoid Insecticide on Performance and Supersedure of honeybee queens

Prof.Dr.Adel R.Hassan

Abstract:
subleathal dosages of Neonicotinoid insecticide were applied in the food of two groups of honey bee colonies (4 colonies each) over one week.The first group were normalcolonies having recenly mated queens,while the second group ofthe colonies were used as feeding colonies for queen rearing.The obtained results indicated that feeding the honey bee colonies with pollen substitute diets or sugar solution having very low dosage of neonicotinoid insecticide greatly decreased egg laying rate of the queens, bees showed agresssive behavior towards their queens and supersedure of these queens occured within very short time.

Chronic exposure of honey bee colonies to sublethal doses of imidacloprid

Hatjina Fani, Charistos Leonidas
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Abstract:
Many times honey bee colonies are exposed to sublethal doses of neonicotinoids for long period, as when they are moved from one treated crop to another, for example from sunflowers to oil-seed rape and from corn to cotton plantations. It is believed that chronic exposure of honey bees in neonicotinoids, is accumulating the stressor resulting to the collapsing or in the better case to the weakening of the colony. Two sublethal doses of imidacloprid (5ppb and 200ppb) were used in a field setup in order to monitor the development and the health of the honey bee colonies for a long period. Imidacloprid was administered in syrup and pollen pastry for almost 2 months and a half. The number of

adult bees and the area of brood (in terms of brood cells) was recorded every 21 days. Samples of bees were also collected for Nosema spore counting and virus analysis. Samples of the stored honey and bee bread were also taken for chemical analysis. After 7 months, most of the colonies treated with 200ppb of imidacloprid are dead and the rest are very weak. The colonies treated with 5ppb of imidacloprid, are significantly weaker than the control colonies. Brood temperature recordings for a month after the end of colony feeding, have shown that the treated colonies were maintaining significantly lower temperatures, especially during the morning hours.

Wing asymmetry of high and low quality honey bee queens

Adam Tofilski, Krystyna Czekonska
Department of Pomology and Apiculture, Agricultural University, 29 Listopada 54, 31-425 Krakow, Poland

Abstract:
Fluctuating asymmetry is often used as a measure of developmental instability of insects. Individuals developing in less optimal environment are expected to be more asymmetrical. We verified a hypothesis that lower quality queens, reared from three days old larvae, are more asymmetrical than higher quality queens, reared from one day old larvae. The asymmetry was assessed using geometric morphometrics of forewings of the queens. Higher quality queens were larger, heavier and differed in wing venation from lower quality queens. The fluctuating asymmetry did not differ between high and low quality queens, however, the smaller queens were significantly more asymmetrical than larger queens.

Body weight of newborn queens from *Apis mellifera macedonica* population

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Abstract:
Eighty-two newborn queens from 2 genotypes of *Apis mellifera macedonica* population were assessed for their body weight in spring 2009 and 2010. In both years the queens were reared under identical conditions by using same starter colony. At the time of the measurement, queens' age varied from 2 to 8 hours. For measurement of the queens we used electronic analytical balance with precision of four decimals. By One-way ANOVA the year as a factor was identified to have a significant effect on the queens' body weight. The mean values of the queens' body weight in 2009 ranged from 0.1518 to 0.2445 g and in 2010 ranged from 0.1315 to 0.2376 g. Additionally, we did not identify significant difference of the queens' body weight between the studied genotypes.

Sublethal doses of imidacloprid decreased protein content and proteolytic activitity in honeybees

Jerzy Wilde[1], Regina Frączek[2], Maciej Siuda[1], Beata Bąk[1]
[1]Apiculture Division, University of Warmia and Mazury, [2]Biochemistry Department, Faculty of Biology, University of Warmia and Mazury

Abstract:
The aim of the assay was to compare the level and activity of proteins in bees fed with sublethal doses of imidacloprid. We also observed the behavior of the bees and assessed the level of contamination with neonicotinoid in food supplies collected in the bee colonies after the feeding.

Bee colonies were randomly assigned to 3 experimental groups, 12 colonies in each group. In early August 2013 the bees were fed with bee syrup (Apifortuna) and pollen pastry made from fresh pollen loads and inverted syrup Apifortuna in the ratio 2.5:1.4. The colonies were given 5.5 kg of the liquid food and 0.3 kg of the pastry in two portions. Colonies from group I (control group) were given food free from

imidacloprid, while the food administered to colonies from group II and III was contaminated with 5 and 200 ppb of imidacloprid, respectively.

Prior to winter feeding, we collected a sample of honey, bee bread and pastry from each colony in order to assess the residues of imidacloprid. Bee samples for testing were collected when the whole administered food was eaten, that is on August 20 and then again after 7 weeks, at the beginning of October. From each colony 20 bees were taken from the upper surface of honey combs in the central part of the nest.

We did not notice differences in the pace of feeding on the administered food among the colonies from individual groups. Neither did we observe any typical symptoms of pesticide poisoning or other illnesses. In October we removed from the experiment 4 colonies from group III (200 ppb) that were unlikely to overwinter. We attribute the weakening of these colonies to queen losses due to feeding on contaminated food. Till the end of January 2014 the wintering of all the remaining 32 colonies proceeded successfully.

The level of protein in bee tissue extract was related to the dose of imidacloprid ($F_{2,479}$=118.6, p=0.0000), the time that passed from the contact ($F_{1,479}$=388.7, p=0.0000), and the significance of interactions between these factors was confirmed ($F_{2,279}$=6.0, p=0.0027).
The highest average protein level (7.8 mg/ml) was observed in bees fed with food without imidacloprid. A significantly lower level of protein was found in bees fed with food contaminated with 5 ppb of imidacloprid (6.9 mg/ml), and with 200 ppb of imidacloprid (5.5 mg/ml).

The average activity of proteolytic enzymes in bee tissue extract was related to the dose of imidacloprid ($F_{2,479}$=63.8, p=0.0000). The time that passed from the contact did not have any effect on protein activity ($F_{1,479}$=0.7, p=0.3973), and no significant interactions between these factors were observed. ($F_{2,279}$=1,6, p=0,1952).

The highest average enzyme activity (0.27 U/mg) was found in bees fed with food free from imidacloprid. A significantly lower proteinase activity was observed in bees fed with food contaminated with 5 ppb of imidacloprid (0.20 U/mg), and with 200 ppb of imidacloprid (0.18 U/mg).

Effects of pesticides on queen health

Geoff Williams, Aline Troxler, Peter Neumann, Laurent Gauthier
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Abstract:
Reports from both Europe and North America suggest that poor queen health is a major driver of honey bee colony mortality. Although numerous environmental stressors are known to influence the health of honey bees, it is not known which are important to queens. Here we exposed developing honey bee queens to field relevant doses of clothianidin and thiamethoxam, and monitored numerous indices of queen quality, including life span, emergence mass, spermatheca-stored sperm number and vitality, flight number, and overall mating success. Results will be discussed.

Evaluation of queen susceptibility and virus transmission routes for CBPV

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Abstract:
Colony loss is often linked to varroa transmitted viral pathogens. However, queen mortality is frequently considered a factor, too. We conducted an experiment to test the susceptibility of queens to Chronic Bee Paralysis Virus, a disease mostly known to affect the workers in honey bee colonies. We found a similar disease progression in the queens compared to worker bees following infection. In a second experiment we showed a different disease progression pattern among three different ways of virus transmission, where queens with forced contact to symptomatic worker bees acquired the disease more easily

than queens in contact with symptomatic worker bees, but with a chance to receive food from healthy bees. Symptomatic worker bees may transmit sufficient active CBPV particles to the queen through trophallaxis, to cause an overt infection. Behavioural mechanisms seem to exist to minimise this route of transmission.

Relationships between oriental hornet Vespa orientalis and some races of honeybees and their descendant hybrids

Abd Al-Fattah, M.A. and Ibrahim Y.Y*

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Abstract:

Relationships between oriental hornets, *V .orientalis* and both of Carniolan and Italian honeybee colonies and their descendant hybrids included numbers of hornet attack, its capture efficiency, capture rate and defensive efficiency of those honeybee races or hybrids against this enemy during two successive seasons,2011-2012. There is no significant difference between the mean numbers of hornet attack Carniolan colonies, (0.53 ± hornet / 3 min. / col.) and those attacked Italian ones, (0.62 ± hornet / 3 min. / col.), the Italian bees were attacked by a higher numbers of hornets than Carniolan ones, especially from 3rd week of September. Also the Italian bees exposed to a significant aggressiveness attack from hornet than Carniolan bees from the middle of October to the end of season. A positive correlation was found between the numbers of hornet attack and its capture efficiency on Carniolan (0.2088) and Italian (0.2946), honeybees. Concerning the hornet predation rate throughout daytime, it could be noticed that this feature was significantly strong from 1000 hr. to 1800 hr. with a peak value at 1600 hr for the Carniolan and Italian bees. The mean capture rate was determined by 0.21 bees / hornet at any time of its active season.

Output of the COLOSS.2 Training School

"Queen Quality and Breeding"

Introduction

For two and a half days, 22 scientists (1 from Canada, 1 from Egypt and 20 from Europe), gathered together to discuss subjects relevant to the quality of queens and / or drones as well as the common activities of RNSBB group, as task force within COLOSS for breeding and conservation.

Sunday, 2nd March 2014

The whole of the first day was devoted to presentations by the participants as follows. All abstracts are included in the proceedings of the training school.

- *Malgorzata Bienkowska*, Dariusz Gerula, Beata Panasiuk, Pawel Wegrzynowicz:* Weight of honey bee queens and its effect on the quality of instrumentally inseminated queens - Multiple use of queenless colonies in mass honey bee queens' rearing
- *Cecilia Costa*, Raffaele Dall'Olio:* Assessment of success of isolated mating of *Apis mellifera siciliana*
- *Anrée Rousseau, Radu-Ionut Apreutese, Marie-Odile Benoit-Biancamano and Pierre Giovenazzo**: Testing a sublethal dose of imidacloprid and a formic acid varroa treatment on drone development and fertility
- *Aleš Gregorc*:* Testing queens from commercial queen rearing apiaries
- *Dubravko Kezić, Janja Filipi, Marica Maja Dražˆ*, Nikola Kezić:* Flow cytometry analysis of drone sperm
- *Fani Hatjina*; Leonidas Charistos*: Chronic exposure of honey bee colonies to sublethal doses of imidacloprid
- *Adam Tofilski*, Krystyna Czekonska*: Wing asymmetry of high and low quality honey bee queens
- *Jerzy Wilde*, Regina Frączek, Maciej Siuda, Beata Bąk*: Sublethal doses of imidacloprid decreased protein content and proteolytic

activitity in honeybees

- *Geoff Williams*, Aline Troxler, Peter Neumann, Laurent Gauthier:* Effects of pesticides on queen health
- *Esmaeil Amiri*, Marina Meixner, Ralph Büchler, Per Kryge*r: Evaluation of queen susceptibility and virus transmission routes for CBPV
- *Abd Al-Fattah, M.A. and Ibrahim Y.Y** : Relationships between oriental hornet *Vespa orientalis* and some races of honeybees and their descendant hybrids

Monday, 3rd March 2014

During the first part of the second day a broad discussion took place on the joint experiment investigating the effect of neonicotinoids on queens and drones, as a continuation of what was already started by Fani Hatjina and Jerzy Wilde in 2013. The discussion included the methodology to be used, the protocol of the experiment, the types of the neonicotinoids and the rest of the parameters that could affect the fate of the experiment. A list of involved members/teams and their specific task and role will be circulated by Fani as soon as possible among the interested parties.

During the afternoon of the second day, more specific discussions took place related to membership issues of the RNSBB group.

Tuesday, 4th March 2014

The last day of the workshop, was devoted to discussions on new common projects. Previous and new ideas were presented, possibilities of funding and the continuation of our work for BEE BOOK was also discussed.

A manual for beekeepers will be one of the priorities of the group, as well as a 'Wing reference data set'. A common experiment for 'Varroa Sensitive Hygiene' could also take part in collaboration with the 'Varroa' task force of COLOSS.

The next RNSBB meeting will be held in Murcia within the COLOSS meeting, prior to EurBee, and the next one in Kirchain, with the exact time-frame still to be determined.

General conclusion

The three days spent on discussing issues related to the quality of queens and drones, as well as breeding activities of the COLOSS members, were very productive and fruitful. New ideas, new common experiments started to be organized and more scientific interactions are taking place.

We feel that the workshop was successful and fulfilled its purpose.

Fani Hatjina,

For the local organizers.

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