Action FA0803

Proceedings of

COLOSS Workshop WG2+4 "Diagnostic Surveys"

August 25-26, 2011
Hotel Palace Belgrade/Serbia

UNIVERSITY OF BELGRADE
Belgrade - SERBIA

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A. Agenda

BELGRADE - COLOSS WORKSHOP "DIAGNOSTIC SURVEY" AGENDA			
day	**time**	**activity**	**place**
24.08. (Wednesday)		Arrival and transfer	Hotel Palace
	16,00	Registration	
	19,30	BOAT CRUISING and dinner on the river banch restaurant	
25.08. (Thursday)	08,00	Registration	Hotel Palace
	09,00	Session 1: **Survey Methods** Questionnarie Surveys Abstract Presentations (5 min.) -Maja Drazic "Regional Variation of Honeybee Colony losses in Croatia" -Ivana Tlak Gajger "Survey About American Foulbrood Diagnostic and Eradication Procedures in Croatia" Diagnostic Surveys (Presence/Prevelance) Abstract Presentations (5 min.) -Tamas Csaki "Introduction of Some Methods and Use of Lab and Field Work with Honey bees in Hoarding Cages" -El-Niweiri "African Foulbrood Diseases Distribution and Diagnosis" -Plamen Petrov "Morphometric Characteristics of Honeybee Populations *Apis mellifera* in Bulgaria and Their Infestation with *Varroa destructor* and *Nosema* spp." -Elif Güzerin "The Prevalence of AFB in Turkey 2010-2011 period" -Fani Hatjina "Diagnostic Survey for the Impact on Predation and Honey Bee Colony Losses, caused by the invasive species *Vespa veluntina* in France" Plenary Talk "Sampling Methods for Diagnostic Surveys" Dr. Aslı Özkırım	
	11,30	Cofee break (Cofee, biscuits...)	
	12,00	Session 2: **The Importance of Diagnostic Surveys for Monitoring Global Colony Losses and Pathogen Interactions(Annual mapping of COLOSS like WHO, FAO)**	
	14,00	Lunch, served on a buffet basis	
	15,00	**Session 3: GEI and WG 4 experiments** Plenary Talk "Effects of genotype and environment for disease relevance – preliminary results from the genotype-environment experiment of WG4 Dr. Marina Meixner &Dr. Cecilia Costa" Abstract Presentations (5 min.) -Malgorzata Bienkowska "Varroa mite population in Genotype-Environment Interaction Experimental Colonies in Poland"	
	17,30	Departure to Sremski Karlovci (BeekeepingMuseum...)	
	19,30	Tour of town	Sremski Karlovci town
	20,15	Dinner	
	22,30	Return to the hotel	

26.08. (Friday)	8,00 9,00	Registration **Session 4: The Role of Environmental Differences between Countries for Using Standardized Surveys** Discussion	Hotel Palace
	11,00	Cofee break (Cofee, biscuits...)	
	11,30	**Session 5: Data Analysis of Diagnostic Surveys** Discussion	
	14,00	Lunch	
	15,00	**Session 6: Discussion- Conclusion**	
	17,30	Cofee break (Cofee, biscuits...)	

Registration on site is required:
Registration fee: 40 €

Workshop information

CONFERENCE LOCATIONS
Hotel Palace **Belgrad/SERBIA**

CONTACTS FOR FURTHER INFORMATIONS	
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A. List of Subjects

B. List of Abstracts

Varroa mite population in genotype-environment interaction (GEI) experimental colonies in Poland

Małgorzata Bieńkowska[1]*, Jerzy Wilde[2], Beata Panasiuk[1], Dariusz Gerula[1]

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Three experimental apiaries for evaluation of GEI experimental bee colonies were established in 2009 in Poland. They were set in different areas of Poland (south-east, central and north). Bee colonies with queens belonging to 8 populations were placed in the apiaries: CarG GR1 from Pulawy, Poland, CarP Kortowka from Olsztyn, Poland, MacB Macedonica from Bulgaria, Mel P Augustowska from Poland, CarC from Croatia, Car V Veitshöchheim from Germany, CarK from Kirchhain, Germany, CarL from Lunz, Austria.

Varroa mite infestation level was monitored in the summer of 2010 in colonies with four different methods:

- washing bees - number of mites per 10 g of bees (spring and late summer)

- brood checking - number of mites in cells with sealed brood (5x5 cm piece of comb) (late summer)

- brood traps - number of mites in cells with sealed brood of a comb that left in a colony after brood withdrawn (July)

- natural mite fall checked in the spring time.

In bee samples from 0 to 15.8 mites per 10 g of bees were found. It is very surprising that in most of experimental colonies in both brood samples, small pieces of combs and in brood traps, no mites were found. In only few colonies single mites were present. Also some mites were fallen on hive bottom board. No correlation was stated between all these methods and mites presence in experimental colonies.

African foul brood disease? Distribution and diagnosis

El-Niweiri, M.A.A.

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Honeybees in tropics suffer more injury and mortality from pests than from diseases caused by invisible organism. However the repeated importation of honeybees in Africa may spread novel diseases eradicating non-adapted regional wild populations. Moreover little is known if any about indigenous honeybee brood diseases in Africa. In this review study we surveyed the foul-brood diseases in Africa. Most of African honeybee diseases were found to be exotic. The distribution of theses disease is common in north and West Africa, but very rare in East Africa. The only brood disease found to be endogenous in Africa is a disease caused by the bacterium *Serratia marcescens* . The infection of this disease reduced the strength of the infected colonies by about 61% and increased their superseding by two and 3 folds as compared to healthy colonies. Due to the first report of this disease in Africa particular in Sudan in addition to the presence of semi-serious between it and the American foul brood and European foul brood in terms of symptoms and damage to colonies, it could be considered as a third foul brood disease, that could possibly be called Africa foul brood disease. The paper also addresses the history of the emergence of this disease, and its diagnosis.

Survey about American Foulbrood diagnostic and eradication procedures in Croatia

Ivana Tlak Gajger*, Denis Cvitković, Zlatko Tomljanović

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Apiculture, as well as diagnostic laboratories for honeybee diseases, has long tradition in Croatia. American Foulbrood (AFB) is a serious honeybee disease present on this region and spread worldwide, and it causes significant losses to apiculture and economy in general. In this survey losses of honeybee colonies caused by AFB, procedures after determination of *Paenibacillus larvae* at bee yards and opinions about early detection of disease we handed out a questionnaire at the annual beekeeping convention 2011.
In total, 216 beekeepers responded to the questionnaire and only 0.46% of them think that honeybee losses at their bee yards were caused by AFB. Also, answers about procedures after determination of *P. larvae* in samples of honeybee brood from their honeybee colonies were very divergently. 64.35% of beekeepers automatically will burn their infected colonies; 8.33% will control the disease by shaking methods; 44.91% will contact official doctor of veterinary medicine. It is interesting that 1.39% of examined beekeepers said that they don't know how to recognize signs of AFB in honeybee colonies. Also, 2.31% of beekeepers still use the antibiotics in AFB treatment, regardless that procedure is prohibited in EU and Croatia. The justification in this is possible development of resistance to used chemotherapeutic agents, masking of disease, relapses, as well as harmful antibiotics residua or their secondary metabolites in apian products. In the same time, a considerable number of beekeepers (84.97%) mean that is necessarily to conduct a routine diagnostic of AFB.
Based on results of questionnaire we can conclude that with better communication between beekeepers and doctors of veterinary medicine, correct and fast diagnostic procedures and good organized improvement and eradication of disease we can reduce AFB prevalence to minimum level.

Regional variation of honeybee colony losses in Croatia

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Colony losses are reported regularly in recent years in Croatia. During winter 2010/2011 surveys on colony losses were collected from 1904 beekeepers from the whole territory of Croatia. Surveyed beekeepers wintered 178523 and lost 18887 colonies. Of those surveyed, 780 beekeepers did not report losses, while 59 beekeepers had losses exceeding 50%. Significant differences in colony losses were observed among regions, with the highest average losses in mountain (14,44%), and lowest in Mediterranean region (5,76%). Average colony losses in continental Croatia were 12,25%. When the losses were compared between different areas regarding agriculture intensity, the losses were 6,47; 8,94 and 13,16% in non-intensive, semi-intensive and intensive agriculture region, respectively. The differences in colony losses between agricultural regions are statistically significant (p<=0,05).

Effects of genotype and environment for disease relevance – preliminary results from the genotype-environment experiment of WG4

Marina Meixner[1*)],Sreten Andonov[2)], Stefan Berg[3)]; Malgorzata Bienkowska[4)],Maria Bouga[5)], Ralph Büchler[1)], Leonidas Charistos[6)] Yves Le Conte[7)], Cecilia Costa[8)], Winfried Dyrba[9)], Roy Francis[10)], Fani Hatjina[6)], Evgeniya Ivanova[11)], Nikola Kezic[12)], Hrisula Kiprijanovska[2)], Seppo Korpela[13)], Per Kryger[10)], Beata Panasiuk[4)], Hermann Pechhacker[14)], Plamen Petrov[15)], Aleksandar Uzunov[2)], Jerzy Wilde[16)]

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The international experiment to estimate the importance of genotype-environment interactions on honeybee vitality and colony losses was started in July 2009 with 621 colonies, involving 18 strains of European honeybees in 16 test locations spread all over Europe.
During the experiment, the health status of these colonies is continuously monitored, in addition to survival and colony performance. Each colony is regularly checked for any disease symptoms, and samples are taken and

analyzed for the Varroa infestation level, the presence of Nosema spores, determination of Nosema species, and the infection with viruses.
Preliminary analyses of the data available so far show strong location effects but nogenotype effect for the Varroa infestation level. However, in the case of Nosema, highly significant effects were observed for both, test environment and genotype, and also for interactions between them. The virus analyses are still in progress.
The data will provide insights into the occurrence of diseases in relation to environmental conditions and genotype-environment interactions

Sampling Methods for Diagnostic Surveys

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The epidemiology of honey bee diseases should be revealed on the base of countries in order to be understood the pathogens' direction and strategy in the world. That's why diagnostic survey methods are crucial for epidemiologic studies. Diagnostic surveys must be done according to certain rules to obtain accurate results. The sampling method chosen is the main factor for diagnostic surveys and this choice directly affects the results of surveys. Especially in big countries and regions have different climates and geographic forms, survey requires a true choice of the sampling methods. Moreover, epidemiologic surveys can be significant by only statistical analysis. Therefore the convenient sampling method for the study is required to give a reliable statistical analysis results. There are many sampling methods for diagnostic surveys. Basic sampling methods can be classified as follows: Simple random, Stratified, Systematic, Cluster and Two Stage method respectively. Although some of these sampling methods seems to be easy and useful for every kind of epidemiologic study, they are not available for using them in different situations. In case the climate affected the pathogens' prevalence in the big country where it can be observed different climates in different regions, the sampling method for one area is not able to represent the entire area. But some sampling methods were created for big and inaccessible regions. For these reasons, sampling methods should be chosen according to the aim of the study and then statistical analysis determined with selected sampling method. This situation provides reliability and quality to the results of diagnostic survey.

The Outbreak of American Foulbrood in Turkey 2010-2011

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American foulbrood (AFB) is a serious disease causes several colony losses in all over the world. AFB has been observed in the range of 9-13% in all regions of Turkey for many years. Diagnostic survey methods have been used for the detection of the disease in every year. In 2010-2011 period, the causal agent of AFB, *Paenibacillus larvae* prevalence is determined in a high rate, 17% . For sampling, systematic sampling method were used. All samples were analysed for AFB in the laboratory. The infection level and the epidemiology of the disease were revealed by statistical analysis. The results show that the prevalence is getting high in two cases: leaving antibiotic use illegally (the first reaction of bacterial growth) and climate change(warm weather conditions induce vegetative growth of *P. larvae).*

Diagnostic survey for the impact on predation and honeybee colony losses, caused by the invasive species *Vespa velutina* in France

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The studies of colony losses in Europe are mainly focused on pathogens (i.e. *Varroa destructor*, *Nosema ceranae* and *Nosema apis* etc) or on external causative agents such as intoxications, nutritional malfunction, environmental factors etc. However, little attention is paid to the impact of predation, mainly by invasive species like the Asian hornet *Vespa velutina*.
Vespa velutina was found to have been introduced in southwest France during 2004. Since then it is expanding rapidly, covering one third of the French territory and causing severe losses to honeybee colonies. During 2010 it was also found in North Spain and is expected to colonize other European countries. Its eradication seems potentially impossible. Since European honeybees never had the chance to interact and coevolved with this predator, their defense is considered as rather insufficient. Using video monitoring and a data acquisition system, designed not to disturb the natural behaviour of honeybees (pray) and hornets (predator) we investigated whether honeybees succeed defending their colonies against hornets and how prey and predator interact.The studied parameters were the flight activity of honeybees and hornets as well as the predator-pray interactions during the whole predation season. Furthermore, using a combination of traps applied in the apiaries or adjusted at beehive bodies, we evaluated the predation pressure in terms of colony losses during the whole predation periods. Finally, using questionnaires we tried to evaluate the impact of hornets' predation on colony losses.
The results indicated that honeybees' defence in France is still ineffective, confirming previous results obtained during simulative artificial attacks with entrapped hornets. Additionally, colony losses are severe in some areas and years, reaching a maximum percentage of 70%. The beekeepers at the infected areas ranked the predation by *Vespa velutina* as the main causative agent of colony losses.

Morphometric characreristics of Honeybee populations *Apis mellifera* (L) in Bulgaria and their infestation with *Varroa destructor* and *Nosema* Spp.

*Plamen Petrov[1], Kalinka Gurgulova[2], Rumen Valchovski[3] ,Nikolay Petkov[4]

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It has made morphometric performance for six exterior signs of 409 samples of bee populations from different regions of Bulgaria. Bees were tested for infestation with Varroa destructor and Nosema spp. Both Nosema spp. - N. apis N. ceranae were differentiated. The study sought the correlation between racial purity and infestations of the investigated colonies. The results showed that morphometric studied bee population as a whole belonged to the local for Bulgaria honeybee.
It was found that 10.62% from samples collected in the first stage were infected with Varroa and in 62.80% of samples were present Nosema spp. Only one sample (0.77%) was positive for N. apis, while N. ceranae was detected in 99.23%. In the second stage of investigation 34.16% of samples were infested with Varroa and Nosema spp. were established in 7.92%, as the species was presented exclusively by N. ceranae.

Introduction of some methods and use of lab and field work with honey bees in hoarding cages

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There has been made several conclusions at the COLOSS Work Shop (Bologna) for Standardized methods for honey bee rearing in hoarding cages.
In the studies with honey bees under laboratory and some field conditions the use of hoarding cages is always a question whether the type of cages are affecting the results.
In the cases where the cages are reused after studies on toxicity the cages could contain residues from the previous cycles. After studies on pathogens the material of he cages are made off affects the type and sufficiency of disinfection or sterilization.
Even during inserting the bees in the cages the need for using CO2 could be a factor affecting the bee's mortality. These factors and specially the practicality were taken into account when we tried to design a universal and disposable cage for standardizing honey bee studies. In our talk we intend to propose some methods and use of lab and field work with our hoarding cages.

C. List of participants

Name-Surname	Country
1.Marina Meixner	Germany
2.Maria Bouga	Greece
3.Dariusz Gerula	Poland
4.Aleksandar Uzunov	FYRUM
5.Alexandros Papachristoforou	Greece
6.Tamás Csáki	Hungary
7.Jerzy Wilde	Poland
8.Aslı Özkırım	Turkey
9.Per Kryger	Denmark
10.Ralph Büchler	Germany
11.Fani Hatjina	Greece
12.Evgeniya Ivanova	Bulgaria
13.Grażyna Topolska	Poland
14.Vincent Dietemann	Switzerland
15.Dragan Bubalo	Croatia
16.Aygün Yalçınkaya	Turkey
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18.Leonidas Charistos	Greece
19.Hrisula Kiprijanovska	FYRUM
20.Anna Gajda	Poland
21.Plamen Petrov	Bulgaria
22.Mogbel El-Niweiri	Sudan
23.Lidija Svečnjak	Croatia
24.Elif Güzerin	Turkey
25.Małgorzata Bieńkowska	Poland
26.Nikolaos Emmanouil	Greece
27.Beata Panasiuk	Poland
28.Kalinka Gurgulova	Bulgaria
29.Maja Dražić	Croatia
30.Forsi Mohammad	Iran
31.Geoffrey Williams	Switzerland
32.Erkay Özgör Fouat	Turkey
33. Cecilia Costa	Italy