Action FA0803

Proceedings of

7th COLOSS Conference
Prevention of Honey Bee Colony LOSSes

26-28 August, 2011
Hotel Palace Belgrade/Serbia

UNIVERSITY OF BELGRADE
Belgrade-SERBIA

Dear colleagues,

On behalf of the local organizing committee, Mića Mladenović and Ljubiša Stanisavljević, I would like to welcome you all to the VIIth COLOSS Conference and MC meeting in Belgrade, Serbia.

In addition to the local organizers, many other COLOSS members have worked tirelessly to make events in Belgrade run smoothly, including Fani Hatjina, Asli Ozkirim, Bach Kim Nguyen, Aline Fauser, Geoff Williams, and others.

A thank you goes out to all attendants and abstract submitters, and especially to our invited speakers – Jamie Ellis and Baldwyn Torto – for agreeing to speak to us.

We have many important topics to discuss, such as future collaboration with the new EU reference laboratory, *BEEBOOK* logistics, and upcoming workgroup events, so I trust we will spend our time in Belgrade wisely. But please, do not forget to enjoy the city!

I am looking forward to seeing you all at our events in Belgrade, which are made possible by financial support granted by COST via the Action FA0803 COLOSS.

Sincerely,

Peter Neumann, Action Chair
Bern, Switzerland, Monday, 22 August 2011

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A. Agenda

Agenda
7th COLOSS Conference
Belgrade, Serbia, 26-28 August 2011

Friday, 26 August 2011

Time	Activity	Location
18:00-20:00	**Executive Committee Meeting** with MP Chauzat (EU reference laboratory) and M Haury (COST)	Hotel Palace - BANQUET
20:00-21:30	Sightseeing of the Belgrade city center and fortress	Belgrade Old Town
21:30-	Free time	

Saturday, 27 August 2011

Time	Activity	Location
8:00-9:00	Registration	Hotel Palace - PANORAMA
9:00-9:10	**Welcome and organizational matters** by M Mladenović and P Neumann	
9:10-9:30	COLOSS finances and politics by P Neumann	
9:30-10:30	Colony Collapse Disorder: myth or reality in Africa? by B Torto	
10:30-11:00	Break (coffee and biscuits)	
11:00-12:00	The COLOSS *BEEBOOK*: a manual of honey bee research methods by JD Ellis	
12:00-14:00	Buffet lunch, **poster session** & **business lunch of *BEEBOOK* editorial board and senior authors**	Hotel Palace - RESTAURANT
14:00-16:00	**Separate Workgroup 1, 2, 3 & 4 meetings** (**detailed *BEEBOOK* planning**, 2011 workshop reports by local organizers, and 2012 planning & budgets)	Hotel Palace - BANQUET, PANORAMA, APP. 111 & TV, respectively
16:00-16:30	Break (coffee and biscuits)	Hotel Palace - RESTAURANT
16:30-17:45	**Separate Workgroup 1, 2, 3 & 4 meetings** (2011 workshop reports by local organizers, and 2012 planning & budgets)	Hotel Palace - BANQUET, PANORAMA, APP. 111 & TV, respectively
17:45	Departure for Radmilovac, Experimental farm of Faculty of Agriculture - University of Belgrade	Radmilovac
18:15	Radmilovac tour	
20:00	Return to Belgrade Hotel Palace	
21:00	Social dinner with music and artistic programme	Hotel Palace - PANORAMA

Sunday, 28 August 2011

Time	Activity	Location
9:00-9:10	Opening remarks by M Mladenović and P Neumann	Hotel Palace - PANORAMA
9:10-9:30	WG1 overview by chairs	
9:30-9:50	WG2 overview by chairs	
9:50-10:10	WG3 overview by chairs	
10:10-10:30	WG4 overview by chairs	
10:30-11:00	Break (coffee & biscuits)	
11:00-13:00	**Management Committee Meeting COST Action FA0803** and open discussion	
13:00-14:00	Lunch	Hotel Palace - RESTAURANT
14:00-onwards	Transfers of participants to transport and optional programs	

Registration of 50 € required on-site

Conference location:

Hotel Palace, Topličin Venac 23, 11000 Belgrade, Serbia
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Local organizers:

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B. List of Abstracts

The COLOSS *BEEBOOK*, a manual of honeybee research methods

James D. Ellis[1*], Vincent Dietemann[2], Peter Neumann[2]

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During the COLOSS workshop *New Molecular Tools* organised in Bern in May 2009, the COLOSS membership decided to work toward internationally recognised methods in honeybee research. We believe such standardization will allow comparison between data on honey bees being generated in various labs internationally and permit us to better understand the problem of bee losses globally. From the Bern discussions emerged the concept of an online working platform dedicated to the creation of the *BEEBOOK*, a manual of honeybee research methods modeled after the widely used book *Drosophila: A Practical Approach*. The *BEEBOOK* will be edited by a 3 member team who solicited senior authors for each proposed *BEEBOOK* chapter. The senior authors are in the process of choosing a team of individuals who are intimately familiar with the subject matter of a given topic. Once all authors of a given section have completed writing the section, the senior author will be responsible for submitting the final version via the online platform. The *BEEBOOK* editors will send the chapters to referees for peer review, much like a refereed manuscript. The reviewed sections will be returned to the senior authors for correction. Once appropriate corrections are made, a section then will be accepted as a completed for the *BEEBOOK* and published online in their respective chapter. After all chapters are received and published online, they will be assembled into a hard copy of the book which will be made available through a solicited publisher. Upon completion, we believe that the *BEEBOOK* will be a reference tool used by honeybee and other researchers globally. We anticipate the inclusion of the following chapters/subject material in the *BEEBOOK*: (1) hoarding cage protocols, (2) *in vitro* rearing of bees, (3) molecular techniques, (4) chemical ecology protocols, (5) biochemical protocols, (6) toxicology protocols, (7) behavioral protocols, (8) estimating colony strength parameters, (9) equalizing colonies for field research, (10) miscellaneous laboratory techniques, (11) GIS technology and honey bees, (12) estimating colony losses, (13) techniques associated with bee pests/pathogens (including American foulbrood, European foulbrood, nosema, varroa, viruses, fungi, endosymbionts, small hive beetles, tracheal mites, and tropilaelaps), (14) instrumental insemination of honey bee queens, (15) queen production, (16) characterization of breeding populations and ecotypes, and (17) other topics as identified by the senior authors and editorial team.

Colony Collapse Disorder: Myth or Reality in Africa?

Baldwyn Torto

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Globally, honey bees contribute to food production through crop pollination, and ecosystem services for biodiversity conservation, as well as income for millions of people. Recent years has seen a decline in honey bee colonies in the developed world, a phenomenon caused by various factors referred to as 'Colony Collapse Disorder.' These factors include pests, diseases, pesticide exposure, and stresses of modern beekeeping practices such as migratory beekeeping. The question is how much of this decline has occurred in the developing world, in particular Africa, where beekeeping is predominantly practiced by small-holder farming communities using traditional hives. This presentation will discuss what we know and what we need to know about bee health in Africa, with special reference to Kenya, in order to determine whether colony collapse disorder has or is yet to occur in this part of the world.

Study of the major factors threatening the survival of bee colonies *Apis mellifera intermissa* in Algeria

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Bees, in addition to producing honey, pollinate fruit trees and other crops to flowers. Any threat to them, whether from herbicides, pesticides, diseases or parasites therefore have serious consequences not only for beekeeping, but also for agriculture in general.

For several years, many beekeepers have reported deaths at their apiaries of honey bee *Apis mellifera intermissa*. At present, we lack hard data on the causes of these deaths in Algeria.

In order to provide some answers to this problem, we conducted a field study among beekeepers in the Mid-northern Algeria. This study is supplemented by information from cooperative bee of the technical institute of livestock and veterinary services department at the Ministry of Agriculture and the regional laboratories of veterinary medicine.

The analysis results showed bee diseases mainly represented by the varroa, bee poisoning by insecticide treatment, and the degradation of the ecosystem (decreasing flora honey) and climate change. All these factors threaten the native bee and negatively affect the production of honey.

Guided natural selection for *Varroa* tolerance using an island approach

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Western honey bee *varroa* resistance or tolerance seems to be best attained by nature. To naturally select honey bees for *varroa* tolerance we use an annual cycle in which: 1. Colonies including their *varroa* populations are split in spring to produce nukes of about 2500 workers each with one young queen. 2. After mating on an island apiary, with the own population of drones, the colonies develop into mature colonies during summer. 3. Colonies that are in October still too weak to winter are discarded from the experiment. 4. After winter the surviving colonies which develop well and which do produce a frame with drone brood are considered fitting to contribute (both by male and female sex) to the next generation. These will be split again (see 1.). *Varroa* mites are not controlled at all.

Nukes are without sealed brood at about 22-24 days after splitting: then a sample of bees is taken to determine the mite infestation level. During winter, in November through January colonies are brood less, and again the varroa infestation can be estimated.

Infestation in the first two years was typically around 10-15%, but at the end of the third season only 6%. During the first three seasons the loss of colonies exceeded the growth of the (new) summer populations, thus decreasing the population size.

A second group of colonies, offspring from Fries' "Gotland" experiment, without *Varroa* control since 2007, is increasing strongly, and infestation at the end of the season is around 6%. Since young colonies can grow fast during summer, they can outcompete *varroa*. Infestation typically about halves from July to December.

Preliminary results of honey bee colony losses in Austria 2010/2011

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Since 2007/2008 we survey the winter losses of honey bee colonies in Austria, distributing the COLOSS questionnaire on meetings, via the internet and a beekeeping journal. So far, losses were between 9.3 and 16.2%, with remarkable differences among regions and years. The latest figures show that 24,451beekeepers in Austria kept 307,303 colonies in 2010. This is more than reported in previous years, because for the first time the 60.000 colonies kept by 248 professional beekeepers are also included. Up to now (May, 13th), we received 353 questionnaires representing 9394 colonies. The total loss from this sample population was 15.8% (95% confidence interval: 12.0-19.6%). Again, some regions suffered total losses of up to 27.1% whereas others experienced lower losses (10.5%). According to the beekeepers, 6.1% of all surveyed colonies 'disappeared' without dead bees in the colony, a symptom indicating any form of depopulation syndrome. Winter losses made up the majority of colony losses in our surveyed period: Of 7648 colonies kept by 334 operations in summer, a total loss of 2.2% (95% confidence interval: 0.6-3.8%) was reported by beekeepers. The number of colonies lost can be compensated by beekeepers to maintain the population size of honey bee colonies in Austria. Still, some operations and also regions experienced losses that require considerable efforts for compensation. We will present this data at the working group 1 workshop and also at the COLOSS conference in Belgrade.

Preliminary results of the international genotype-environment interaction experiment of WG 4

Ralph Büchler[1*], Stefan Berg[2], Malgorzata Bienkowska[3], Beata Panasiuk[3], Yves Le Conte[4], Cecilia Costa[5], Winfried Dyrba[6], Maria Bouga[7], Fani Hatjina[8], Leonidas Charistos[8], Plamen Petrov [9], Evgeniya Ivanova [10], Nikola Kezic[11], Seppo Korpela[12], Per Kryger[13], Hermann Pechhacker[14], Aleksandar Uzunov[15], 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.

The common test protocol considers colony survival, bee population in autumn, spring and summer, productivity, swarming, gentleness, hygienic behavior and the infestation with Varroa, Nosema and viruses. No chemical treatments against Varroa and diseases were applied since 2010. In most test apiaries, all brood combs were withdrawn once during season in order to reduce the level of Varroa infestation.

23,8 % of the colonies were lost until the end of May 2010 and another 16,2 % were lost until the end of January 2011. Besides problems with the queens (23,1%), most losses were linked with symptoms of Varroa disease (25,6%), Nosema or defecation (7,9%) or weakness and robbery (5,8%). No clear symptoms were observed in 32,6 % of the cases.

The data analysis shows a strong influence of the test location on the strength of the colonies at all control intervals. However, we can also observe highly significant differences between the different strains and highly significant interactions between genotype and environment.

Regarding the Varroa infestation of bee samples, significant effects of the location, but not of the genetic origin have been observed. However, in the case of Nosema infection, the test environment, the genotype, and interactions between them all show highly significant effects.

The tested genotypes clearly differ in their honey productivity, gentleness and swarming tendency which can at least partially be explained as a consequence of different breeding intensity

for these classical selection characters. However, it is important to note that even regarding these characters highly significant genotype – environment interactions can be observed.

To sum up our primary results, we can state a high relevance of interactions between honeybee genotypes and different environmental conditions within Europe. Obviously, the genetic adaption of honeybees to a specific environment influences its population dynamics, health status, and productivity. Consequently, the conservation of European honey bee diversity and the support of local breeding activities should be pushed forward.

Honey bee winter losses in England, 2007-10

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Recent media coverage on honey bee colony losses, especially those attributed to "Colony Collapse Disorder" in the USA, has focussed attention on colony losses elsewhere. Many countries, including the UK, lacked hard data on what "normal" colony losses, particularly those which occur in winter. This made it difficult to assess whether "abnormal" losses were occurring. Consequently, in 2008 the British Beekeepers Association, which represents some 20,000 amateur beekeepers, mainly in England, began an annual survey of a random sample of its members. The survey questions intended to allow the calculation of winter losses included those standard questions developed by Working Group 1 "monitoring and diagnosis" of the international COLOSS (Prevention of COLony LOSSes) network, but also covered treatments used against the parasitic mite *Varroa destructor*. The results of four years surveys will be discussed, together with other data collected by the Food and Environment Research Agency National Bee Unit (NBU), the UK government agency responsible for honey bee health in England and Wales, and other sources.

Varroosis diagnosis through brood symptoms and mite counts

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The parasitic mite *Varroa destructor* Anderson & Trueman (Acari: Varroidae) is the most detrimental pest of the honey bee *Apis mellifera*. Adult female mites are phoretic and feed on adult worker and drone bees. They leave their adult hosts to invade brood cells occupied by mature bee larvae just before worker bees seal the cells with wax. Colonies infested by *V. destructor* will eventually suffer deleterious effects. It is observed that the honey bee brood exhibit typical damage symptoms, such as scattered brood nest and crippled bees when the varroosis is at later stage of development.

The aim of this work was to evaluate the brood symptoms in correlation with mite detection on adult bees and on sticky boards. We compared three groups of eight colonies. Different modalities were applied to the groups: untreated group (control), Apivar®-treated group, and over-treated group. Four complete visits occurred between October 2009 and April 2010. At each visit, adult bees were sample from each colony. Varroa were dislodged from bees by washing the bees with alcohol. Honeybee population was evaluated on each colony frame. Symptoms on brood and on adult bees were recorded. Varroas were counted on sticky boards every two weeks to look at the natural fall for the control group or falls due to acaricide treatments for the two other groups.

Detection of pyrethroid resistant mites in Ireland

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Varroa destructor, an ectoparasite of the honeybee was first identified in Ireland in the late 1990s. For the past 13 years, beekeepers have been using Bayvarol, a chemical insecticide almost exclusively to control the *Varro*a mite. In August 2010, the Department of Agriculture Food and Fisheries carried out a pilot survey to determine the efficacy of the Bayvarol and to establish if resistance was developing. Preliminary data confirms the presence of resistant mites, but their geographical distribution has not been documented. As part of the present Apicultural Research programme, we aim to monitor the spread of resistant mite using proteomic analysis. This method offers the possibility of examining the protein profile of an organism and sequencing the amino acid constituents of individual proteins. Previous work has been employed to examine and identify the proteins secreted by a mite of humans, *Demodex folliculorum*. Proteins will be extracted from flumethrin sensitive and resistant mites and resolved by 2-Dimensional electrophoresis. Progenesis software will be employed to identify changes in the expression of key proteins. These will be excised from gels, trypsin digested and analysed by LC/MS. The identity of proteins will be established and those showing altered expression in *Varroa* will be sequenced in order to establish if point mutations are responsible for resistance.

As resistance is developing in Ireland, it is crucial to identify alternative treatments which are effective and reliable under Irish conditions. At present, the only alternative product to pyrethroids (Bayvarol) is Apiguard. However, the efficacy of Apiguard gradually decreases below 15°C and in Ireland in late Autumn daily ambient temperatures regularly drops below this threshold. Thus, research is on-going with the aim of developing an integrated pest management system which is both effective against the *Varroa* mite and which can be incorporated in to Irish legislative system.

Progress Report WG3: Vitality of honey bees and in-vitro rearing

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One of the potent tools in investigating the impact of environmental stress on honey bees is the in-vitro rearing of larvae. Young larvae are removed from the colony and can be treated with various doses of pesticides, other substances from the environment, pathogens or can be exposed to physical stressors. The application of this method was previously discussed in a work shop in Austria (2010) and also mediated in a short-term-scientific-mission (STSM). In November 2011, a follow-up workshop will be held in France.

Another topic of interest of work group (WG) 3 is honey bee colony vitality, which was addressed at work shop in Wageningen (Netherlands) in June/July 2011. Here the establishment and validation of methods to assess honey bee vitality and health at individual and colony level was discussed. One aim is the development of a "toolkit" to determine the colony vitality as result of parasite infestation, feed / environment, diseases and (chronic) exposure to pesticides.

A further cooperation project about influence of electromagnetic fields on the development of honeybees is started.

How to get rid of EFB in Norway

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Until 2010 the last verified incidence of European foulbrood caused by the bacteria *Melissococcus plutonius* in Norway dates back to 1980 when it was found in one apiary. EFB is listed as a notifiable disease in Norway and suspicion of EFB should be reported to the Norwegian Food safety Authority. In July 2010 EFB was suspected in several apiaries and samples analyzed at the Norwegian school of veterinary science verified the presence of *M. plutonius* in the samples. The goal of the strategy chosen to fight the disease has been to eradicate *M. plutonius* in Norwegian apiaries. Testing positive for *M. plutonius* in or more colonies/apiaries entails destruction of all colonies and destruction/disinfection of all beekeeping equipment. In 2010 about 3000 colonies belonging to 45 beekeepers were destroyed. Economical compensation paid to the beekeepers for the sanitation in 2010 was about 1000-1.500 000 €. In 2011, by May 11, *M. plutonius* has been found in the apiaries of 8 beekeepers. These 8 beekeepers have about 400 colonies and are all located within the area of the outbreak in 2010. In 2010 colonies were inspected for clinical symptoms and a brood comb were sent for analyses using a standard PCR technique on larvae. In 2011 a more sensitive qPCR on wax debris has been used which allows sampling on the levels of apiaries. Whether the strategy to minimize the distribution and prevalence of EFB is successful or not will be known within the next few years. It is not known whether *M. plutonius* in Norway belong to the same clone, or if several genetically different strains are involved. A number of Norwegian *M. plutonius* isolates will be sequenced and provide answer on this question. The virulence of the Norwegian *M. plutonius* strain (s) will be evaluated and compared with other high and low virulent strains.

The course of *Nosema ceranae* infection in Poland

Anna Gajda, Grażyna Topolska*

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Nosema ceranae is known to infect bees in most European countries, as well as most countries outside Europe. The disease pattern itself seems to vary greatly depending on climatic differences. For instance, while in Spain *N. ceranae* causes most of the bee losses, in Sweden it does not seem to be a serious problem. In this study we wish to determine the pattern of the disease in Polish climatic conditions. In the experimental apiary at WULS we are observing colonies with pure *N. ceranae* infection, which seems to be ongoing since at least 2007. We are also examining bees from two other apiaries with mixed *Nosema* infections (*N. ceranae* infection predominates over *N. apis* infection). The examination in both cases is done using PCR methods (to establish *Nosema* species) and also light microscopy (spore counts and percentage of infected bees). To date (4 years since we confirmed *N. ceranae* infection in the WULS apiary), most of the colonies are still alive and with confirmed presence of *N. ceranae.* This differs greatly from the situation in Spain, where most of the infected colonies die by the end of the second year of infection. The examination of two additional apiaries (outside WULS) showed that in 33% of the colonies in which in 2009 *N. apis* or *N. apis + N. ceranae* were detected, in 2010 only *N. ceranae* was found. In 2009, in the outside apiaries, the mixed infections were more common (detected in 63% of colonies), whereas, in 2010, mixed infections were found in only 20% of examined colonies. In 50% of the colonies free of *Nosema* in 2009, in 2010 *N. ceranae* or *N. apis + N. ceranae* appeared. Investigation of dead bees collected from the hive bottom boards at the end of two winters (2009/2010 and 2010/2011) suggests, that in 64% of the colonies the level of infection increased, while in 26% it decreased and in 9% it did not change. However, the investigations carried out at the National Veterinary Institute in Pulawy, of dead bee samples from Polish apiaries where the losses exceeded 10%, has not yet revealed any correlation between bee losses and the level of *Nosema* infections in honeybees.

Influence of genetic variability of bee workers on vitality of bee colonies-preliminary results

Dariusz Gerula*, Paweł Węgrzynowicz, Małgorzata Bieńkowska, Beata Panasiuk, Wojciech Skowronek

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The aim of the research was to determine the influence of genetic variability of worker bees in bee colonies on their productivity and vitality.

Three lines of Carniolan bees were used in the study. Experimental queens were bred from single Cariolan queen. Drones for semen collection were taken from 30 different colonies of all three breeding lines. Queens were instrumentally inseminated. experimental queens were inseminated with semen collected from drones from a single colony (SCS-Single colony semen) or with mixed semen collected from several colonies - (MS-Mixed semen).

Queens, from both experimental groups, were introduced into colonies in two apiaries "S" and "W", differing in abundance and the date of flow. In 2009, total of 102 colonies were prepared for winter. SCS colonies were stronger after the first winter, while after the second winter they were weaker in comparison to MS colonies.

There were no differences stated between the number of colonies and the degree of infestation with Nosema spp in both experimental groups in both years. No indication was found that the degree of infestation of bees was related to the losses of colonies. Autumn infestation with Varroa destructor was slightly lower in the MS group. Hygienic behavior test was performed in June. Bees from SCS group cleaned slightly more cells (average 44%) than MS colonies -41%.

Measurements of brood was performed in each colony every 3 weeks' time starting from mid-May. MS colonies had more brood than SCS group.

Colonies from the MS group produced more honey (mean 11.8 kg), compared with SCS group-10.3 kg however in "W" apiary MS colonies stored 20% more honey than SCS colonies.

The effects of diesel exhaust pollution on stress responses in forager honey bees

Robbie D. Girling[1,*], Sergio Regufe Da Mota[1], Joanne Bailey[2], Guy M. Poppy[1], Tracey A. Newman[2]

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Global honey bee (*Apis mellifera*) populations continue to be in sharp decline, but to-date the reason for these colony losses are not fully understood. A variety of stressors are thought to contribute to losses, including pests and pathogens, insecticides and poor forage resulting from current agricultural practices. No single key factor has been identified as causing the global scale losses that have been observed. It is likely that the reason for population declines are multi-factorial and that a variety of stressors are acting synergistically to reduce bee fitness. However, those stressors of most importance may be inconsistent and vary between different environments, precluding the possibility of isolating any individual cause. Many environments in which honey bees forage also contain a variety of other pervasive and ubiquitous environmental pollutants, which have to-date been ignored as potential stressors on bee health. One such substance is diesel exhaust pollution, a major pollutant in urban areas and along roadways. Diesel exhaust pollution is comprised of gaseous and particulate fractions, and is known to result in inflammatory responses in mammals. We have investigated the effects that acute exposure to diesel exhaust pollution has on inflammatory responses in the central nervous system of foraging worker honey bees. Successful returning forager bees were collected and exposed to a controlled acute dose of diesel exhaust pollution. We then investigated inflammatory responses in the central nervous system of these bees and compared them to those of a group of unexposed forager bees. To do this we employed the use of immunohistochemistry and Western blot analysis techniques. We will discuss the implications that these results may have on the health and fitness of honey bee colonies.

A case study of acute toxicity in honeybee colonies induced by acaricide coumaphos

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Abnormal bee behavior was observed 4 hours after coumaphos CheckMite+ strips had been inserted between two brood frames in each colony. Bees started to leave the hives, flew extensively around the hives, clustered on the front hive wall and dropped down in the grass. Workers also gathered there in smaller clusters and were dying in the surrounding of the treated hives with extended wings, and curved, shortened and tremored abdomens. Bees were also clustering on the back hive doors and walls in the inner side of the hives. Brood combs in the lower hive compartment were scared, and dead workers were found on the hive bottom board. Treated colonies were reduced for approximately 1/3 of adult bees. Workers were sampled from the bottom board, brood and honey compartments and grass in front of the hives. We performed GC analyses, and quantify coumaphos by gas chromatography- electron capture detection (GC-ECD) with the limit quantification (LOQ) of 30 ppb. Coumaphos was found in workers sampled in brood compartment, honey compartment and in front of the hives at the levels of 1771, 606 and 514 ppb (µg/kg) respectively, which did not exceed the oral dose LD50. Nevertheless, it would be possible that some bees were exposed to much higher pesticide doses, as miticides do not distribute evenly within the bee colony. Bees can receive 3.2 µg of coumaphos in colonies treated with CheckMite+ (Haarmann et al., 2002) and in the previous study we showed that 2 µg of coumaphos already affects food transfer (trophallaxis), foraging activity, homing and learning ability of workers which could interfere with colony fitness. The interactions between pesticides used for crop protection and mite stresses are likely contributing factors. This supports a hypothesis that no factor alone is theoretically responsible for the dramatic worker mortality of CheckMite+ treated honey bee colonies. Pesticide treatments can induce toxic effects in honeybee colonies and reduce bee population. Application of acaricides in honeybee colonies should thus be applied with a great consideration.

Status of WG 2 "Pest and Pathogens"

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Honeybee pests and pathogens play a major role in colony losses. Therefore, this topic is of great interest to the members of COLOSS. This is mirrored in the fact that as of April 28, 2011 a total of 266 COLOSS members from 54 countries indicated Working Group 2 "Pests and pathogens" as their primary interest (100 are members WG 2 only). To meet the challenges brought about by coordinating such a big group we introduced a second vice chair last year (Asli Özkirim from Turkey) and defined two main goals: (i) applied research activities and (ii) basic research activities. Thus we will be able to take advantage of the size of this working group and install new and fruitful collaborations that can provide new insight to our understanding of various pests and pathogens in relation to colony losses.

Two interesting STSM projects granted for 2010 took place since the last conference: Claudia Dussaubat (France) worked in Spain on 'Standardizing laboratory procedures for collaborative research on *Nosema* spp. and pesticide interactions on honey bees', and Eva Forsgren (Sweden) worked in Germany on 'Pathogenesis of *Melissococcus plutonius* in individual larvae using FISH technique' . Three STSMs have been granted for 2011 and will take place in the near future: (i) Aygun Yalcinkaya (Turkey) will work at the Laboratory of Zoophysiology, Ghent University in Ghent, The Netherlands, on "Genotyping methods of *Paenibacillus larvae*: Preliminary studies of strain-race relationship between *Paenibacillus larvae* and *Apis mellifera*"; (ii) Svjetlana Vojvodic (Denmark) will learn a "Laboratory protocol for measuring hygienic behavior, detection and removal of diseased brood" at the INRA Avignon Research Centre, Honey Bee Biology and Protection Laboratory in Avignon, France; (iii) Cansu Ozge Tozkar (Turkey) will work at the Centro Apícola Regional in Marchamalo, Spain, on "Experimental infection of honey bees by *Nosema ceranae* and *Nosema apis*".
All STSMs have a high component of knowledge and technology transfer between laboratories and countries.

During the current period (from last COLOSS conference) three WG2 workshops have been scheduled. Two already took place until May 2011: A workshop on "Varroa, viruses and standardisation of methods" in Bern, Switzerland, NOV 1-4, 2010 and a workshop on "The future of brood disease research – guidelines, methods and developments" in Copenhagen, Denmark, APR 10-12, 2011. A workshop on "Diagnostic surveys" is scheduled for AUG 25-26, 2011 in Belgrade, Serbia and will be organized together with WG4.

In the forthcoming meeting we will shortly present the outcomes of our workshops and STSMs and plan future WG 2 activities. We will also try to present scientific breakthroughs and progress which furthered our understanding of bee diseases and related colony losses. We will also try to extract ideas from the participants how to achieve our goal 'Improved basic knowledge on bee pests and pathogens to improve our understanding of the complex phenomenon of pest- and pathogen-associated colony losses'.

Imidacloprid residues on honeybee, honey and pollen from colonies placed on cotton fields

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Imidacloprid is one of the neo-nicotinoid substances shown to have detrimental effects on honey bees even after their exposure to sub-lethal doses. Honey bees foraging on fields like cotton, that are derived from seeds dressed in imidacloprid, take sub-lethal doses of imidacloprid in amounts of 2-7 ppb. In order to determine the residual prevalence of imidacloprid on the bee tissue, honey and pollen, a number of honey bee colonies were placed in treated cotton fields, as well as in non treated fields. The determination of imidacloprid was achieved by Liquid Chromatography coupled to Mass Spectrometry operating in tandem mode (HPLC-ESI-MS/MS). Extraction and cleanup were based on a modified QuEChERS method, involving Solid Phase Extraction (SPE) step for the purification of analyte from the matrix (bee, honey, pollen) interference. Briefly two transitions were selected; one for the identification (256 to 175 amu) and one for quantification (256 to 209 amu) of imidacloprid. The Limit of Detection (LOD) of the analytical method was 1.26 ng/g. Imidacloprid was not found on the bees tissues, honey and pollen from untreated fields, however, it was detected in the amounts of 8.79 ng/g in honey bee tissue, 5.68 ng/g in pollen and 7.42 ng/g in honey 25 days after the colonies were placed in treated cotton fields.

Genetic variability of honey bee origins used in the GEI experiment: mitochondrial DNA analysis

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One of the main goals of COLOSS WG 4 is to establish a common protocol for the discrimination of honey bee populations. In Europe, a host of methods are used to determine the subspecies origin of honey bees. In WG4 different methods are currently applied to analyze samples of the colonies that are part of the common GEI experiment; mtDNA data will be combined with the results from microsatellite, isoenzymic, classical and geometrical morphometric analyses.

These data will contribute to the documentation of the genetic origin of each colony involved in the common experiment and to the establishment of a published and accessible reference database that will be of value to scientists and apiculturists working in the field of European honey bee biodiversity and conservation. Preliminary results from the sequencing analysis of the mtDNA control region D-loop, will be presented.

The parasitic mite *Varroa destructor* in the primitive and Langstroth hives

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Each member of the honey bee colony may be clinically or genetically burdened by a wide variety of pathogens. Regardless of the honeybee colony where belong, can significantly affect the development and reproduction of the entire community, and they are responsible for significant colony losses. One of the most important pests is the parasitic mite *Varroa destructor* alone, and as a vector of various viruses (Israeli acute paralysis virus, Kashmir bee virus, Sacbrood virus, Varroa destructor virus 1, Deformed wing virus, Cloudy wing virus ...). Therefore, the colony size, volume of the hive and daily control of the brood development, may affect significantly on greater or lesser presence of mites in the brood and at the older bees.

In our experiment, we monitored the development of honey bee colonies in the standard LR hive, in the modified LR beehive for organic beekeeping, and in the primitive hives adapted for organic beekeeping. The mean value of dropped *Varroa* mites was highest in the strongest colonies that are housed in standard LR hives: 428.3, then in the modified LR hives: 247, 8, and in the primitive hives: 183.2 and the lowest in the most primitive hives: 66.2. On the basis of the other statistical values we can concluded that the *Varroa* mites were most developed in the strongest colonies.

Honey bee situation in Belgium

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For the season 2009-2010, the total mortality rate in Belgium was 27.56%. The problematic of honey bee mortality in Belgium is multifactorial and the mean honey bee mortality causes have been identified (*Varroa destructor*, viruses, quality and quantity of food before and during the winter). To measure the honey bee colony mortality, the standardized questionnaire level 1 and 2 which is used by the participating countries in the COLOSS project was completed. Belgian scientists are conducting the monitoring of the Working Group 1 and they are measuring the total honey bee colony mortality rate for the winter 2010-2011. In the same time they are evaluating the solution proposed by the Belgian government and they are estimating the impact of the advices formulate the previous year to the beekeepers.

Colony Losses in Turkey 2010-2011

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From March to the end of April we have conducted a web based survey on colony losses in the different regions of Turkey 2010-2011. The questionnaire is based on the basic COLOSS questionnaire that was the result of the meeting in WG1 in Amsterdam January 2010.

Data from the survey will be processed during the first week of June and results presented at the meeting in Belgrade.

AFB and EFB - early detection bee brood disease, monitoring survey 2010 in Republic of Srepska, BiH

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During the period from March till November 2010 in voluntary program for early control American foulbrood we tested 1053 honey brood samples. We used Celle method for early detection AFB spores.

We find that from tested samples we registry spores of *Paenibacillus larvae* in 6, 74% samples. What did surprise us was that with same method is possible to isolate easily *B. alvei*, which is declared as secondary microbiot in EFB disease. We find them more frequently than AFB in 11, 2% samples.

The question that we want understand is about two things: First, we faced that this method have some limitations, in some cases when is clinical form of AFB present in a particular hive we did not find spore at detective level from them. What can be reason for that? Second, if it is accepted, today's opinion that EFB is complicated with *B. alvei*, can we use that indicator as a valuable marker for EFB presence in hive. If we cannot do that, what is the reason that we do not have that bacterium in other hives?

Those issues about host parasite relation in hive homeostasis and health status have to be discussed. Clinical case definition for EFB is still not recognized worldwide and we have to research real role of different biota in that complex. Viral load at brood level is good starting point to know more.

Evaluation of colony losses in Israel 2008-2011

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Since 2008 we implement two approaches to evaluate the local levels of the colony losses in order to characterize the potential causal factors that include:

1. a survey, among beekeepers and,
2. regular monitoring of specific hives during the year.

Detailed questionnaires were distributed among the beekeepers in 2008, 2009 and in 2010 and are planned for 2011. In 2010 two questionnaires were handed, one dedicated to evaluate winter losses using a level 1 questionnaire developed by COLOSS working group 1, and a detailed questionnaire to evaluate annual losses. In addition, hive monitoring was conducted in 2009 and 2010 and included about 110 hives each. While, in 2010, the emphasis was on the impact of *Varroa* infestation on the outbreak of diseases and colony collapse, in 2011 it is on the impact of *Nosema* evaluating 6 sites with 250 hives in total.

Over the years, our survey data, represented 34-50% of total colonies but only 9-15% of the beekeepers and indicated that the overall level of colony losses was below 20%. It appears that the high levels of losses (above 40%) occur among small beekeepers (with operation size below 100 hives) and are not associated with migration or pollination services. In the 2011 survey we plan to use improved questionnaires (for winter and annual loss evaluation) based on the conclusions of working group 1.

Hive-monitoring in 2010 indicated that increase in *Varroa* levels was accompanied by higher virus incidence, in particular Deformed wing virus and *Varroa destructor* virus-1, followed by brood and adult disease and subsequent collapse of the hives. Another important factor observed was infection with *Nosema* that developed towards autumn. We hope that this year experiments will clarify the role of this pathogen in colony losses.

Vitality of honey bee colonies as a result of pollen diversity and prevalence of Nosema spp. DWV and ABPV

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To assess the impact of pollen diversity on the vitality of honey bee colonies, 10 colonies were places in a region having high diversity of pollen and 10 colonies having low pollen diversity. The test period was May – September 2011. The vitality parameters of the honey bee colonies were: mean colony hemolymph vitellogenin, number of bees, number of sealed brood cells and prevalence of DWV, ABPV and Nosema spp. The outcome of the study was:

- Pollen diversity differed significantly between the regions;
- Higher pollen diversity in spring resulted in a higher fraction of mean colony vitellogenin in spring;
- A higher mean colony fraction hemolymph in June results in significant more sealed brood in July;
- In September, the colonies in the region having a high pollen diversity had significant more bees and less sealed brood than the colonies in the low pollen diversity region, showing that the transition from summer to winter population in the high pollen diversity area started earlier;
- In all colonies DWV and *Nosema ceranae* were prevalent;
- In colonies having a relative high vitellogenin fraction more *N. ceranae* spores and ABPV were detected.

Varroa treatment in the Netherlands 2007-2010

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Beginning at 2007 monthly data on Varroa treatment were collected from Dutch beekeepers as part of the yearly Monitor Winter losses. Since the identity of the beekeepers is known, comparisons could be made between the years concerning the association between winter losses and the effectiveness of the varroa treatment. The varroa strategies were evaluated with a time model. A strong association was found between the month of treatment and winter losses two winters later. This is explained as timely treatment in year 1 results in low mite loads in spring of year 2 with the effect of lower losses in the following winter.

The Bee Informed Partnership

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The Bee Informed Partnership is an extension project that endeavours to decrease the number of managed honey bee colonies that die over the winter.

Since the winter of 2006 – 2007, overwintering colonies in the US have died in large numbers. Affected beekeepers span the entire spectrum of the industry: migratory beekeepers to stationary beekeepers; and commercial beekeepers, part-time beekeepers, to backyard beekeepers. Migratory and stationary beekeepers alike have, on average, lost 30% or more of their overwintering colonies over the last several years. These losses are unsustainable. If they continue, they threaten not only the livelihoods of beekeepers who manage bees, but the livelihood of farmers who require bees to pollinate their crops.

This project will adapt the tools developed by human epidemiologists to study complex human diseases (such as cancer or heart disease) to study honey bee colony health. However, this project will be slightly different than traditional "community health" initiatives in a couple of important ways:

1. Its focus will be to identify management practices that keep colonies alive (rather than just looking for factors that increase the risk of mortality)
2. Findings will be shared rapidly, transparently, and in ways that will enable beekeepers to make informed individualized decisions

At its core, the Bee Informed Partnership is motivated by the conviction that beekeepers, when presented with beekeeper-derived data that objectively shows which management practices worked and which did not, will adopt the more successful practices.

The Effect of Herbal Toxicity of Rhododendron Plants on Colony Losses

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In the last few years, colony losses has attracted great attention as a global problem in all over the world. There are lots of factors considered as causes of colony losses: new pathogens and pathogen interactions eg. *Nosema ceranae, viruses and Varroa and other pathogen co-infections*; and also pesticides eg. *neonicotinoids.* Although, there are many collaborative investigations for finding causes of colony losses under the Coloss-Cost Action, some pieces of puzzle are still missing. Honey bees forage on a variety of plant species throughout their life. It is well documented that the plants' attractiveness for bees correlated with the sugar content of the nectar or the nutritive value of pollen. Herbal toxicity is one of the unconsidered causes of losses. There are many reports of toxic nectar. Most of these studies focused on honey bees or humans poisoned by honey made from nectar of specific plant. Presence of allelochemicals especially alkaloids in nectar and pollen cause toxic effect to the bees. We have frequently detected nectar poisoning from the last year in some of the samples that taken from collapsed colonies. *Rhododendron* pollens were found dominantly in the intestines of those bees by microscopic investigation. There are five different Rhododendron species in Turkey: *R. ponticum, R. luteum, R. caucasicum, R. smimovii, R. ungernii*. Honey produced from *Rhododendron ponticum* called "Mad Honey" because of the grayanatoxin that found in the nectar of this plant. This plant grows in Black Sea Region especially on the mountains. Rhododendron honey is very valuable medically because of its trace elements and phenolic compounds contents. There are a lot of researches about toxic effect of this honey to humans. But no paper about the toxic effect of the Rhododendron nectar to the bees is reported. It is known that locally adaptive bees are not affected from Rhododendron nectar and can produce mad honey. On the other hand, colony losses have been occurred in new introduced honey bees to this area for the lack of adaptation to the toxin. Due to the new introduced colonies are the result of migratory beekeeping movement, toxic nectar producing plants should be considered especially countries where the migratory beekeeping is common. In case especially at the high temperature , climate change causes the toxin become more intense because of evaporating of the water in the nectar content. The high intension might affect the local bees as well.

C. List of participants

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