Action FA0803

Proceedings of

6th COLOSS Conference

Prevention of Honey Bee COlony LOSSes

Hacettepe University,
Ankara, Turkey

5.-6.09.2010

Dear colleagues,

On behalf of the local organising team, I would like to welcome you to the 6th COLOSS conference at the Hacettepe University of Ankara.

Appreciation is addressed to all the people who have helped to organise and conduct this meeting. In particular, it would have been impossible without the tireless efforts of Dr. Asli Özkirim and her team and Dr. Fani Hatjina.

I would also like to thank all contributors for submitting their abstracts, which I hope will stimulate rewarding discussions. Please keep in mind that the focus of this meeting will be the standardization of our approaches in the form of a BEEBOOK and to plan the activities until the next conference.

Financial support is granted by COST via the Action FA0803 COLOSS.

I am looking forward meeting all of you, and hope you will enjoy this conference.
Yours sincerely

Peter Neumann, Action Chair
Bern, Switzerland, Monday, 30 August 2010

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A. Agenda

Time	Programme
4th September 2010 (Saturday)	
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20:00-	**Social dinner at Yeşil Vadi Restaurant**
5th September 2010 (Sunday) – Hacettepe University Mehmet Akif Ersoy Hall	
08:00 – 09:00	Registration
09:00 – 12:00	**General Assembly and plenary session**
09:00 – 09:15	Welcome and organizational matters by Asli Özkirim & Peter Neumann
09:15 – 9:30	The future of COLOSS by Peter Neumann
09:30 – 9:45	Honeybee health in Africa by CWW Pirk
09:45 - 10:00	Honeybee health in Asia by Panuwan Chantawannakul
10:00 - 10:30	***Coffee break with snacks***
10:30 – 10:45	Honeybee health in South America by Fancy Rojas
10:45 – 11:00	Honeybee health in North America by Dennis vanEngelsdorp
11:00 – 11:15	The BEE BOOK by Vincent Dietemann
11:15 – 11:30	BEE DOC & STEP by Peter Neumann
12:00 – 13:30	***Lunch***
13:30 – 14:45	Separate WG meetings: Hacettepe University Dept. of Biology reports of workshops by local organizers, planning & budget for 2011
14:45 – 15:00	***Coffee break with snacks***
15:00 – 16:30	**Separate WG meetings: Hacettepe University Dept. of Biology planning & budget for 2011**
16:30 – 16:45	***Coffee break with snacks***
16:45 – 18:15	Separate WG meetings: Hacettepe University Dept. of Biology focus on the BEE BOOK
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20:00 -	**Social Dinner at White House Restaurant**

Registration on site is required:
Registration fee: 10 €

6th September 2010 (Monday) – Hacettepe University Mehmet Akif Ersoy-Hall	
09:00 – 10:30	**General Assembly with overviews by WG leaders (progress, work shops, plans for 2011)**
09:00 – 09:15	WG1 by Romée van der Zee and/or Yves Le Conte
09:15 – 9:30	WG 2 by Annette Bruun-Jensen and/or Elke Genersch
09:30 – 9:45	WG3 by Karl Crailsheim and/or Ales Gregorc
09:45 - 10:00	WG4 by Cecilia Costa and/or Marina Meixner
10:00 – 10:30	Open discussion
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11:00 - 12.00	**Separate WG meetings: Hacettepe University Dept. of Biology** **detailed plans for 2011**
12:00 – 13:00	General assembly: Hacettepe University Mehmet Akif Ersoy Hall open discussion, BEE BOOK, plans for 2011, budget 2011, wrap up
13:00 – 14:30	***Lunch***
14:30 – 16:00	MC Meeting of the COST Action FA0803
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16:15-17:00	**MC Meeting of the COST Action FA0803**
17:00-	**Small Trip to Beypazarı and Social Dinner II**

Conference information

CONFERENCE LOCATIONS	
Hacettepe University	**Hacettepe University**
Mehmet Akif Ersoy Hall, Beytepe Campus	Department of Biology

CONTACTS FOR FURTHER INFORMATIONS	
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VI COLOSS Conference Local Organizing Committee:
Aslı Özkırım, Aygün Yalçınkaya, Elif Güzerin, Erkay Fouat

Technical support:
Hacettepe University

B. List of subjects

Plenary talks

Overviews by WG leaders

Reports of colony losses- monitoring projects

Pathogens

Pesticides

Genetics-selection

Nutrition

C. List of Abstracts

Plenary talks

The future of COLOSS

Neumann Peter

Swiss Bee Research Center, Liebefeld, Switzerland

First progress has been made with respect to standardizations and evaluation of drivers for honeybee mortality, but we are still far from fully understanding the underlying mechanisms for losses and disseminating adequate mitigation strategies across member countries. Only if we succeed to bridge the gaps between scientists, apiculture and politics will we achieve sustainable progress in the prevention of colony losses. COLOSS as a broad transnational platform not only fosters networking amongst members, but can also serve as a useful tool for the transfer of science into practice and politics. However, in order to fully serve the latter goals, new approaches seem to be required to facilitate COLOSS as an integral part of the global strategy for prevention of colony losses. Indeed, COLOSS has become global with >200 members from currently 49 countries, thereby creating demand for an improved organization. Since, the prevention of honeybee colony losses will also be of concern in the future and the initiation of COLOSS required considerable efforts by several members, it appears worthwhile to maintain the established network structure and collaboration amongst members beyond the COST funding period. Suggestions for future financial support and organization of the COLOSS network will be presented.

Honeybee health in Africa

Christian Pirk

Department of Zoology & Entomology, University of Pretoria

The worldwide observed colony losses seems not to take place in South Africa. Despite that all the major pests and diseases have been reported for South Africa the honeybee population seems to be unaffected. Moreover, the outbreak of AFB does not have the expected negative effect on the South African population. The vital natural population with a high genetic diversity, thus buffering the negative effects of pests and diseases on the population could play a role or alternatively, a lack of data. Therefore, we started a survey filling this potential gap of knowledge and to evaluate the impact of common honeybee diseases in the country.
As expected all the major diseases are present, but it also shows a fundamental lack of identifying skills by the beekeepers. However, local pests, e.g. Capensis, are catching the attention of the industry. This suggests that the other pests and diseases are below the economical threshold, resulting in an indifference of the beekeepers to deal with them. If this conclusion holds, it also suggests that the natural population of African honeybees has traits and features successfully dealing with the diseases compare to its European counterparts. Therefore the African population is the ideal model for investigating the underlying mechanisms.

Bee Health Status in Thailand and Japan

Panuwan Chantawannakul[1], Veeranun Chaimanee[1], Chanpen Chanchao[2], Mikio Yoshiyama[3], and Kiyoshi Kimura[3]

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[2]Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand 10330.
[3] Honeybee Research Group, Animal Breeding and Reproduction Research Team, National Institute of Livestock and Grassland Science, Japan.

In Asian countries, beekeeping industry plays a significant role within the agricultural sector. In Thailand, at least four native honey bee species are found, apart from an introduced species, *Apis mellifera*. Severe cases of bee loss have not been reported. However, during our visit to local apiaries in 2009 in order to conduct a survey of diseases and pests, few apiaries encountered the colony collapse up to 50%, due to the *Tropilaelaps* and *Varroa* infestations. European foulbrood and Chalkbrood diseases also frequently occur. In addition, honey bees were infested with *Nosema ceranae* and some viruses. In Japan, honey bee population is also of great interest as about half of honey bee hives are used as pollinators in green houses. Even though demands for bees as pollinators are increasing, both honey bee population and number of beekeepers have been declined for years. Recently, the shortage of honey bee colonies has become a serious problem, especially when the occurrence of *Acarapis woodi* was first reported in 2010. Under these situations, massive bee loss is highly concerned. To prevent bee loss both native and *mellifera* bee in our regions, the collaborative network both nationally and internationally in Asia has been established.

Background of winter losses in Chile

Fancy Rojas

Universidad Mayor. Santiago, Chile

Chilean beekeeping activity is mainly developed in the south central region between 32° and 43° south latitude.
Beekeeping in Chile is supported in the native forest and pollination services for fruit production.
The winter average losses are 15-20%. Winter losses have several biotic causal agents (Varroa destructor, Nosema apis, Acarapis woodi) and abiotic (lack of nutrition, weather conditions). Although Varroa destructor is the most important health problem for the Chilean beekeeping, it is not yet possible to assess the real impact of Nosema, which is present a few seasons ago.
The last beekeeping season was affected by a 8,8° MW earthquake, with epicenter in 36° south latitude. In the impact area is located the 85% of the hives. The event took place on February 27th and it caused the death of 5-10% of the beehives and the weakening of the survivor bee families just before the winter season started: after the earthquake it was possible to watch unusual levels of swarms, death of queen bees, less brood, high levels of pillage, among others.

Honey Bee Colonies Losses in the U.S., winter 2009-2010

Dennis van Engelsdorp[1], Jerry Hayes[2], Dewey Caron[3], Jeff Pettis[4]

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The Apiary Inspectors of America (AIA) and USDA-ARS Beltsville Honey Bee Lab conducted a survey to estimate winter colony loses for 2009/2010. Over 22.4% of the country's estimated 2.46 million colonies were surveyed.
A total loss of 33.8% of managed honey bee colonies was recorded. This compares to total losses of 29%, 35.8% and 31.8% recorded respectively in the winters of 2008/2009, 2007/2008 and 2006/2007.
In all 4,207 beekeepers responded to the on-line survey and an additional 24 were contacted by phone. This response rate is orders of magnitude greater than previous years efforts which relied on phone or email responses only (2008/2009 n=778, 2007/2008 n=331, 2006/2007 n=384).
On average responding beekeepers lost 42.2% of their operation, this is an 8 point or 23% increases in the average operational loss experienced by beekeepers in the winter of 2008/2009.
Average losses were nearly 3 times greater than the losses beekeepers reported that they considered acceptable (14.4%). Sixty-one percent of beekeepers reported losses in excess of what they would consider acceptable.
It is also important to note that this survey only reports on winter losses and does not capture the colony losses that occurs throughout the summer as queens or entire colonies fail and need to be replaced. Preliminary data from other survey efforts suggest that these "summer" losses can also be significant. All told the rate of loss experienced by the industry is unsustainable.

The BEEBOOK, progress report

Dieteman, Vincent; Neumann, Peter,

Swiss Bee Research Center, Liebefeld, Switzerland

Prevention of honeybee colony losses requires comparisons of data to be possible internationally. Indeed, data on the quantification of losses in the field need to be measured on the same scale and research on the drivers of losses need to be performed with common methods. Therefore, an international standardisation of monitoring and methods for research is at the core of the COLOSS network. During two previous work shops, the idea of an online BEEBOOK emerged and has been initiated at http://www.coloss.org/beebook.

This online platform will enable interested COLOSS participants to contribute to the development of standards. Here we present how the BEEBOOK platform works and give an update on the work already achieved.

Overviews by WG leaders

Monitoring and privacy considerations- WG1

Romée van der Zee

Netherlands Centre Bee Research, Tersoal, Netherlands

Studying colony losses per beekeeper across the years provides important information. This will only be possible if the identity of the beekeeper is known to surveyor. This raises privacy issues. A resume will be given of state of the art procedures for scientific research. A standard code will be proposed to be discussed in the working groups. This process may lead to a COLOSS code and could be part of the Bee Book.

Status of WG 2 "Pest and Pathogens"

Elke Genersch[1] and Annette Bruun Jensen[2]

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Honeybee pests and pathogens are of great interest and as of August 2010 a total of 93 COLOSS members from 31 countries indicated Working Group 2 "Pests and pathogens" as their primary interest (74 member WG 2 only). Collaboration and team spirit of such a big group is a challenge but it also offers great opportunities for new and fruitful constellations that can provide new insight to our understanding of various pests and pathogens in relationship to colony losses.

Three interesting STSM projects have been granted. All have a high component of knowledge and technology transfer between laboratories and countries: Lidija Svecjnak (Croatia) worked in Bern on 'Interactions between *Varroa destructor*, bacteria and viruses at the individual honeybee level'; Claudia Dussaubat (France) will work in Spain on 'Standardizing laboratory procedures for collaborative research on *Nosema* spp. and pesticide interactions on honey bees', and Eva Forsgren (Sweden) will work in Germany on 'Pathogenesis of *Melissococcus plutonius* in individual larvae using FISH technique' .

During the current period (from last COLOSS conference) several WG2 workshops have been scheduled. One "Nosema diseases: Lack of knowledge and work standardization", already took place from October 19 to 22, 2009 in Guadalajara, Spain and a second one on "Diagnostics in honeybees: From sampling to data analyses" will be held from August 30 to September 1, 2010 in Ghent, Belgium (a joint BEEDOC and COLOSS workshop). Members of WG 2 also actively participated in the workshop "Method standardization for larval tests", Graz, Austria , June 7-9, 2010 and many members attended the combined work shop "The BEE BOOK: Monitoring and Standardization", Amsterdam, The Netherlands, January 18-20, 2010. The first step towards the BEE BOOK with an online working platform has been launched, so now guidelines for honeybee research of various pest and pathogen groups can be made with input also coming from various members of WG 2.

In the forthcoming meeting we will plan future WG 2 activities such as workshops and STSMs and also try to identify those areas in bee pathology where scientific progress is most urgently needed to further our understanding of colony losses. We aim at delivering a list of research priorities combined with first suggestions 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'.

Progress report work group 3 - Method standardization for larval tests

Karl Crailsheim[1], Ales Gregorc[2] and Robert Brodschneider[1]

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[2] Honey Bee Research and Extension Laboratory, University of Florida - Dept. of Entomology and Nematology, P.O. Box 110620, Bldg. 970 Natural Area Dr. Gainesville, FL 32611, USA.

We will give a progress report regarding the research within work group 3 with its main focus on investigating effects of environment (e.g. nutrition) and apicultural practice (hive management) on honey bee health. One of the major achievements in the last months was the work shop "“Method standardization for larval tests” held in Graz, Austria, 7.-9.6.2010. Artificially reared honey bee larvae are an ideal model to apply one or more environmental stress factors, and to investigate not only larval mortality but also the effect of sublethal damage on adults. At the workshop, more than 25 researchers promoted the standardization of used protocols and discussed the quality of honey bees produced by the most commonly used method. Progress was also made regarding technical aspects and trouble shooting. The extension of this method for new applications also was discussed.

Ongoing evaluations of the Coloss WG 4 genotype-environment interaction experiment

Cecilia Costa[1], Ralph Büchler[2], Marina Meixner[2], Malgorzata Bienkowska[3], Beata Panasiuk[3], Fani Hatjina[4], Charistos Leonidas[4], Kokkinis Michalis[4], Evgeniya Ivanova[5], Plamen Petrov[5], Nikola Kezic[6], Maja Drazic[6], Seppo Korpela[7], Aleksandar Uzunov[8], Jerzy Wilde[9]

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9) Apiculture Division, Warmia and Mazury University, Sloneczna 48, 10-710 Olsztyn, POLAND

To test the hypothesis that genotype – environment interactions may have an effect on colony vitality, a comparative field experiment involving different genetic origins of European honeybees is ongoing since July 2009. At 16 different locations all over Europe, 18 different strains and ecotypes of honey bees are being evaluated according to a common protocol, which includes colony development, pathogen levels and hygienic behaviour. In the 6th Coloss conference the first year results from part of the test colonies will be presented.
The experience gained by the test participants will be valuable in the creation of internationally applicable recommendations for scientists and beekeepers involved in honey bee breeding programs.

Posters (by author name)

Preliminary results of colony losses in Austria 2009/2010

Robert Brodschneider[1], Rudolf Moosbeckhofer[2] and Karl Crailsheim[1]

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[2]Austrian Agency for Health and Food Safety, Institute for Apiculture, Spargelfeldstraße 191, A-1220 Vienna, Austria.

So far, 273 Austrian beekeepers have answered the COLOSS questionnaire (version 3.2.10). Most beekeepers responded by mail (44.3%), followed by fax (28.2%) and email (27.5%). In total, these beekeepers maintained 7018 colonies on October 1st 2009. After counting in all bought or split and merged or sold colonies, total colony loss during winter was 13.5% (95% confidence interval: 9.5-17.6%), compared to 9.3% and 13.3% the preceding two winters. The total number of colonies alive on April 1st 2010 did not differ from the number participants kept on April 1st 2009 ($n=270$, $chi^2=0.18$, $p>0.05$, Chi^2 test) but was lower than the number of colonies in October, $n=270$, $chi^2=39.18$, $p<0.05$, Chi^2 test), suggesting that the summer production of honey bee colonies in Austria compensated overwinter losses. This result is presumably biased because only active beekeepers participate in the survey in contrast to beekeepers who abandon beekeeping due to high losses. In Austria, the dates of October 1st and April 1st are convenient to determine overwinter losses, as the majority of beekeepers assessed end and beginning of the foraging season for their bees to be in November (67.3%) and March (67.5%), respectively.

Intra-colony selection for hygienic behaviour in the Dark European honey bee

Norman L. Carreck[1,2], Karin L. Alton[1], Annette B. Jensen[3], and Francis W. L. Ratnieks[1].

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[3]*Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen*
Thorvaldsensvej 40,DK-1871 Frederiksberg C., Denmark.

A key factor in honey bee colony losses worldwide is undoubtedly the parasitic mite *Varroa destructor*, which is now present in all major countries apart from Australia. One reason for continuing losses is that in many areas such as the UK, *V. destructor* has become resistant to the synthetic pyrethroid acaricides which were previously successfully used to control it. Early studies in the USA showed that certain strains of honey bees exhibit so called "hygienic behaviour", which enables them to resist bacterial and fungal brood diseases, and more recently hygienic behaviour has also been shown to confer resistance to *V. destructor*. We are using the novel technique of intracolony selection to enhance hygienic behaviour in the British native honey bee *Apis mellifera mellifera*, with the aim of reducing the reliance on chemical acaricides in bee colonies. Within a colony that has been selected as "hygienic", only a minority of the many patrilines present, corresponding to different drone fathers, may actually exhibit the hygienic trait. Selection for hygienic behaviour on a colony basis will therefore be slow. By using molecular techniques to identify which patrilines actually exhibit hygienic behaviour, and then retaining only queens reared from these patrilines, will thus accelerate the breeding process.

Honeybee diseases monitorization to alert and sanitary risk detection

A. Cepero, C. Botías, , A. Meana, M. Higes R. Martín-Hernández*

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Currently we have launched a research project to develop a system to monitorizing infectious and parasitic honeybee diseases. As well, the study of agrotoxics and chemotherapic in pollen and wax samples is included to provide us data about the contamination levels of both structures from agricultural pesticides and from substances used by beekeepers to treat hives. These substances in foraging areas have been related with pathological problems in bees. This information could also be used as a measure of environmental contamination as has been recently proposed in other European countries. The development of new detection techniques will be also raise.
To develop this Project, a system of passive watch over is planned using the pathological samples received in CAR (Centro Apícola Regional). Also an active survey to get samples around the national territory is currently being carried out on the basis of an epidemiological design.
Results analysis would provide us to know the sanitary status of Spanish beekeeping and also the environmental contamination levels suffering by bees.

Over-wintering of honey bee colonies using different artificial diets

Charistos Leonidas, Hatjina Fani

Hellenic Institute of Apiculture (N.AG.RE.F.), N. Moudania, Greece

Six different artificial diets were evaluating for their efficiency in honey bee colony over-wintering. Each diet was used in a group of 10 colonies. The different diets were: A. commercial Apifonda candy; B. candy with sugar; C. candy with honey; D. candy with sugar and iso-glucose; E. candy with iso-glucose and F. a commercial product containing simple sugars. The total weight of the colonies was recorded before feeding initiation (December 2009) and after 4 months (April 2010). The strength of the colonies (population), the brood area, the honey and the pollen comb area were also recorded three times after the first brood appeared (in February, March and April). In general results show that all diets were efficient in over-wintering the colonies, but best performance was measured in group A and C, while worse performance was measured in groups B and E.

The groups that consumed more were the groups A, B and F. The highest increase in the population was measured in group C, while the highest increase in the brood area was measured in groups A and C. Honey in combs was lower in April than in February in all groups and pollen increase was slightly higher in groups A, B, C and D. Group F weighted 2 Kg more in April than in December while group C had no net difference. As a conclusion we can say that colonies which over-winter with candy made with honey are entering the Spring stronger.

Colony losses in Switzerland: 2009/2010

Jean-Daniel Charrière[a], Robert Sieber[b]

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[b]Swiss beekeeping association Apisuisse, CH-9050 Appenzell, Switzerland

During the 2007/08 winter, we started close collaborations between the Swiss Bee
Research Centre and the Swiss Beekeeping Association. For the 2009/10 winter, via the Swiss beekeeping journals, we looked to increase beekeeper participation in the COLOSS questionnaire. The participating beekeepers were distributed throughout Switzerland and increased from 472 beekeepers in 2008 to more than 650 (4.1% of all Swiss Beekeepers), managing 13'900 colonies in 900 apiaries.
From 1st October 2009 to 1st April 2010, mean colony losses in Switzerland were 20.1% (ranging from 4 - 47% depending on the Canton) and 9.7% of all Swiss colonies surveyed were too weak in spring to develop to a productive colony.
Among the 2807 colonies which died during the 2009/10 winter, 1823 (65%) were found with none or only a few living bees remaining. Generally, except for a few Cantons, the colony losses were heavy and much higher than the former year which was only 8.8% and in the same range as in 2002/03 at 18%, in 2005/06 locally at about 30% according to regional monitoring systems and in 2007/08 at 17%.
The altitude of the apiaries and the age of the beekeepers had no influence on colony losses. The most frequently cited causes by beekeepers were "poor queens", "weak colonies in fall" and "Varroa".

Ants and Varroa: a glue that solves the counts

Chauzat MP, Drajnudel P, Faucon JP.

French Food Safety Agency (AFSSA), Bee disease unit, 105 route des Chappes, BP 111, 06 902 Sophia Antipolis cedex, France.

The ectoparasitic mite *Varroa. destructor* impairs both brood and adult bees causing a serious disease called varroosis. Diagnosis of varroosis relies on the female mite counts and on the observation of damages on the honey bee brood. Female mites can be numbered by washing out infected adult honey bees or by opening brood. A sound diagnosis can be achieved by measuring the natural mite drop on a tray inserted into the hive's bottom board. This technique is also used for measuring the effect of miticide treatments.
Usually, grease from animal origin is used to make sticky trays. This cheap and easy to apply mean is largely used but often attracts consumers. Dead Varroa mites are also good attractants for scavengers such as ants. The feeding action of ants on greased trays often leads to misleading counts of falling mites. Here, we compared two data sets using two different glues to make sticky boards: the traditional animal grease and the glue applied for color traps used in biological control. With this glue, we never noticed any damage on sticky boards cause by ants or any other scavenger during the 7 months-experiment. Varroa counts were repeatable giving consistent results. Moreover, this mean provided more flexible possibilities to read the sticky boards in the laboratory. Since *V. destructor* is a major threat for apiculture, it is important to provide to scientists and beekeepers reliable and repeatable ways for accurately counts of falling mites.

Colony losses in Ireland, 2009/2010

Mary F Coffey, John Breen

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In Ireland, there are approximately 2000 beekeepers managing 20 000 colonies. A high percentage of these beekeepers are members of the Federation of Irish Beekeepers Association (FIBKA) and are actively involved in beekeeping associations at local level. In total, there are approximately 40 local beekeeping associations distributed in different counties throughout the country and their primary aim is to promote beekeeping and to act as educational resources. However, unlike most European countries, data on colony losses is very sparse. On examining the literature the following information was available: prior to varroa, winter colony losses were estimated at approximately 10%, whereas today beekeepers are reporting 15-20% losses. In 2007, a questionnaire was distributed by a local association in Cork and abnormal high losses were reported (53%). In 2008/2009 we carried a pilot project *(n*=35) using the basic questionnaire produced by WG1 and winter losses were estimated at approximately 22%. However, sample size in these three studies was too small to be analysed statistically and could not be used with any reliability to develop disease control strategies which would minimise colony losses. However, it highlighted the importance of carrying out a comprehensible assessment of winter colony losses in Ireland which could be compared and analysed with data from other European countries. Therefore, this season we initiated the first monitoring programme on colony losses using the Basic Questionnaire produced by WG1. The questionnaire was primarily distributed through the local associations. In general, each association held a meeting at the beginning of May and the secretaries distributed and collected the questionnaire on the same evening. Non-members of associations were also encouraged to participate in the survey and thus the questionnaire was made available on the FIBKA web page and in the monthly magazine (An Beachaire) produced by the Federation. In general, the questionnaire was received with enthusiasm and a total of 460 completed questionnaires were returned. The data is presently being inputted and will be forwarded to the COLOSS data base for analyses. It is hoped that this survey will give, for the first time, reliable and comparable data on the winter colony losses being incurred by Irish beekeepers.

What are the reasons for the moderate winter losses in Norway?

Bjørn Dahle

Norwegian Beekeepers Association, Norway

Based on the COLOSS questionnaire 10% of the production colonies in Norway died during the winter 2009-2010. Losses were expected to be higher due to an extraordinary long and cold winter, and because many beekeepers reported that they wintered weak colonies. So what can be the reasons for the moderate winter losses? Beekeepers report that they use organic acids (mainly oxalic acid in fall) alone, or in combination with cutting of drone brood to treat their colonies against *Varroa destructor*. None of the responding beekeepers suspected *Varroa* to be an important source of mortality, although the presence of *Varroa* has been associated with higher winter losses (10.3% vs. 6.3%) in a previous study. In a recent study only 1.3% of 538 honey samples contained spores of *Paenibacillus larvae*. Accordingly only 9 outbreaks of American foulbrood have been reported the last 10 years. In 2009, 78 honeybee samples from 18 beekeepers were analysed for KBV, ABPV, CBPV, SBV, DWV and BQCV. Only DWV and BQCV were found. However, SBV is probably present in Norway. I suggest that the combination of few honeybee pathogens in Norway and a sustainable strategy for treatment of *Varroa* contributes to the moderate winter losses.

Diagnostic analyses of RNA viruses

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Diagnostic analyses of RNA viruses is widely used in honey bee pathology. RNA is very unstable due to the actions of endogenous RNA-degrading enzymes raising the problem of sample conservation until analysis in the laboratory. It is commonly admitted that only the analysis of live bees or bees conserved at low temperatures can deliver reliable results. No single study has investigated the possibility to use dead workers conserved at higher temperatures to get accurate virus diagnostic in the laboratory. Given the short regions of RNA being screened for pathogen diagnostics (generally amplified regions of 100-200 nucleotides), it is conceivable that even degraded RNA will provide a template for precise diagnostics. Here, we evaluated for the first time the impact of the two most convenient sample storage and handling conditions (+4 °C and ambient temperature) for RT-qPCR honey bee virus diagnostics, taking the example of Deformed Wing Virus (DWV) and Black Queen Cell Virus (BQCV), using dead workers. Our data will be useful for the standardisation of sampling methods and will contribute to the Beebook developed in the framework of our COLOSS COST action.

First report of *Apis florea* in Ethiopia

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After the introduction of *Apis florea* in 1985 in Sudan there has been great concern about the spreading of this bee in Neighbor countries. Previous study predicted that *A. florea* will spread into Egypt, Uganda and Ethiopia through the Nile within the next decades. We therefore tested this prediction by conducting a general survey of *Apis florea* along the Blue Nile. We found that *A. florea* crossed the boarder between Sudan and Ethiopia at Al Daim cross border station on the Blue Nile. Moreover the finding of *A. florea* nest is documented in Yarenja refugees' camp near Mankush town in Ethiopia

Results of honey bee health monitoring project in Tuscany (Italy)

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Since 2009, Tuscany Region is supporting a two-years project to monitor honey bee health within provinces of Florence, Arezzo, Siena and Lucca. The project, called "APENET-Toscana", is coordinated by Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, in collaboration with the regional beekeepers associations.
After the first year of activities, it could be possible to realize 57 apiaries inspections to verify the measures used for crop protection in the surrounding areas; 150 hives have been monitored every 3 months to check their health status: mortality, depopulation, strength, presence of diseases and anomalous behaviour of the bees. Samples of bees (556), wax (56), combs (21) and pollen (40) have been taken for analysis to investigate varroa mite, AFB, EFB, *Nosema* spp., viruses, pollen analysis and pesticides.
Average mortality, after 1 year of monitoring activity, resulted of 9,3%. AFB resulted with a prevalence of 3%. It never could be possible to verify cases of acute toxicity due to pesticides. Viruses have been observed within all the investigated apiaries as direct consequence of *Varroa*: DWV (31%), ABPV (24%), CBPV (14%), IAPV (0%), KBV (0%). *Nosema apis* could never be found, while *Nosema ceranae* has been found with high prevalence and variability during the year.

Loss of a colony's population in some apiaries in Iran

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Most of the colonies in centre and south west of Iran showed the high population in current year in early spring, but they gradually lose 50% their population.
No symptoms of any diseases or parasites have observed.
These colonies have enough honey but the deficiency of pollen and protein is evident, and the rate of total protein is under 20%.
The beekeepers believe that the bees leave the hives and never coming back.
In some apiaries the majority of broods (pupae) in colonies are in the reverse form that they can not exit from the cells.
In some regions of the country that the phenomena of Dusting has observed the loss of colony's population is more prominent.

Monitoring honeybee colony losses in Croatian apiaries

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Honeybees are an extremely important part of natural ecosystem because they enhance agricultural productivity and help maintain biodiversity by providing valuable pollination services. In this survey overwinter losses of honeybee colonies we handed out a questionnaire at the annual beekeeping convention 2010. The questionnaire was based on COLOSS guidelines (http://www.coloss.org/). In total, 126 beekeepers responded to the questionnaire and the survey was run on 13228 honeybee colonies which exhibited 13.67% of the winter losses. 35 (27.78%) of the beekeepers reported losses of les than 20%, from which 8 (6.35%) beekeepers reported losses greater than 50%. At the same time, 25 beekeepers (19.84%) reported no losses at all. A considerable number of beekeepers have reported the disappearance of bees from 989 (7.48%) hives, but they found stored food and sealed brood, and they think that bees are probably lost in the form of swarms of die in the field. Beekeepers opinion about potentional reasons for honeybee colony mortality was: *Varroa destructor* (23.01%), bad beekeeping practice (20.80%), queen loss (17.26%), *Nosema ceranae* (10.62%), robbery (10.62%), and lack of food (8.85%), bad weather (7.52%) and unknown reasons (1.33%). We therefore suppose that the main drivers of local colony mortality are pests, pathogens and environmental factors.

Improvement of *Nosema* spore counts for Nosemosis diagnosis

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Nosemosis is a widespread disease affecting adult honey bees. The causing agent is an intracellular parasite belonging to the fungi family. Nosemosis due to *Nosema apis* has been largely studied in the past. Over the last few years, much concern has been given to a new pathogen (*Nosema ceranae*) recently identified in European honey bees. Nosemosis due to *N. apis* is diagnosed through symptoms exhibited by adult honey bees in association with spore counts. At the moment, researches are undergone on the diagnosis of Nosemosis due to *Nosema ceranae* that also take into account spore counts.
In order to improve repeatability and consistency of the spore load evaluation, we compared several spore preparations. The same sample of infected honey bees was differently numbered. Preparation and reading of observation slides were repeated and results compared taking into account the time spent. The relationship between the time consumed for numbering spores and the differences in spore counts let us conclude that the preparation of three slides numbered one time each gave a reliable result.
Individual infestation rates of 10 honey bees were also compared to the mean infestation count. The centrifugation stage reduced the final number of spores when compared to non-centrifuged grinded samples. Sample preparation was studied mixing serums containing known amount of spores with blank serums.

Preliminary results of APENET monitoring for bee health status in Italy

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APENET is a monitoring network of honeybee mortality and colony losses in Italy. The preliminary results of the first year (2009) of APENET's activity is presented. 316 representative samples of adult honeybees from 16 of 20 Italian regions were submitted to the IZS delle Venezie for diagnosis of *Nosema apis*/*Nosema ceranae* infection and for virus detection according to a routine sampling schedule irrespective of any clinical signs. Honey bee crushings were examined by light microscopy for *Nosema* spp spores and used for DNA extraction. PCR was performed for a specific 16S rRNA gene region of *Nosema* spp. For species identification, the PCR products were sequenced and similarity analysis was performed by using BLAST. Honeybee samples were submitted to National Bee Unit, FERA, Sand Hutton, York (UK) for virus detection. *Nosema ceranae* was present in all monitored Italian regions, while *Nosema apis* or *Nosema apis*/*Nosema ceranae* coinfection were not detected. DWV, BQCV, SBV, ABPV and CBPV were detected in Italian apiaries in different combinations. IAPV, AIV and KBV were not detected. Honeybees and wax were also analysed to determine organophosphate, organochlorurate and carbammate pesticides as well as neonicotinoids. APENET preliminary results provide evidence of the endemic presence of *Nosema ceranae* and for the first time systematically investigate the presence of viruses and their geographic distribution in Italian apiaries. At present time pesticide residues appear to be of minor concern.

The effects of pesticides on honeybee (*Apis mellifera*) larvae

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The objective of this research was to determine the effects of pesticides on honeybee larvae. For our first study, we used *in vitro* bioassays to determine LC_{50} values of pesticides on larvae. We found that mycobutanil and glyphosate were virtually non-toxic at the concentrations tested (0 - 400 ppm). Simazine and amitraz were slightly toxic (3163 ppm; 2304 ppm) while imidacloprid and chlorothalonil were more toxic (706 ppm; 351). Chlorpyrifos was the most toxic compound tested (1.6 ppm). Treated larvae showed resistance to fluvalinate and coumaphos at the concentrations administered. In a second study, larval cell death due to pesticides was detected using the TUNEL technique for DNA labeling and Annexin V to detect exposed phosphatidylserine binding to apoptotic cells. Chlorpyrifos-treated larvae experienced 75% cell death; larvae treated with amitraz and coumaphos had <40% cell death. Fluvalinate-treated larvae did not experience heightened cell death. In the final study, pesticide exposure and/or varroa mite parasitism on honeybee larvae led to changes in gene expression, specific for genes that were upregulated, down regulated or neutral. There were significant interactive effects between varroa exposure and pesticide exposure for numerous pesticides and variable genes, indicating a synergism between chemical insults and mite impacts.

Individual oral infection of honey bee workers with a new method

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Honey bee pathology often requires controlled individual oral infection of workers to ensure that each bee receives the same amount of pathogens. The conventional methods are quite labour intensive and time consuming, thereby often compromising sample sizes. Here we present a fast, less labour-intense and cheap novel method, which allows for large sample sizes in honey bee pathology. Freshly emerged workers were individually transferred into standard 1.5 ml reaction tubes. The tubes were then inserted with the lid side into racks and fixed. The tips of the tubes were cut to provide air and an opening of about 0.4 mm. Then, the racks were stored at RT for 2 hours to starve the workers. Each worker was then fed with 5 µl of 50% sugar solution containing a standardised amount of the pathogen(s) (e.g. a Nosema spore solution with 2 x 10^5 spores). To ensure that the sugar water is consumed and not post hoc exchanged between workers via trophallaxis, the racks were stored 30 minutes. Finally, the tubes were opened and workers transferred into standard hoarding cages to monitor worker mortality and infection progress.

Diversity investigation of Bulgarian honey bees based on isoenzyme and microsatellite DNA analysis

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The genetic variability of honey bee populations from approximately thirty different locations in Bulgaria has been studied using isoenzymic and STR Microsatellite DNA analysis. The polymorphism of six enzymic (MDH, ME, EST, ALP, PGM and HK) and seven microsatellite loci have been studied and characterized. All of the studied isoenzymic loci were found to be polymorphic in most of the populations. Three alleles were detected at MDH-1 locus (MDH^{65}, MDH^{80} and MDH^{100}); three allels - at ME locus (ME^{90}, ME^{100} and ME^{106}); six alleles EST 3 locus - EST^{80}, EST^{88}, EST^{94}, EST^{100}, EST^{105} and EST^{118}; three alleles - at ALP locus (ALP^{80}, ALP^{90} and ALP^{100}), four alleles - at PGM locus (PGM^{80}, PGM^{100}, PGM^{114} and PGM^{125}) and three alleles - at HK locus (HK^{87}, HK^{100} and HK^{110}). The percentage calculated of polymorphic loci ranged between 50% and 83%. The observed and expected heterozygosities (H_o and H_e) ranged from 0.142 to 0.244 and 0.219 to 0.296, respectively. Allele frequencies of all loci were used to estimate Nei's (1972) genetic distance, found to range between 0.001 and 0.017. The microsatellite DNA analysis shows that the average number of alleles per locus per populations, ranges from seven to more than twenty and the average observed heterozygosity and gene diversity values for populations studied were reviewed and assessed.

Isolation, preservation, spore production and quality control of the chalkbrood fungus *Ascosphaera apis*

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Chalkbrood is a honeybee brood disease caused by the fungus *Ascosphaera apis*. This fungus can be isolated and grown *in vitro*. Monthly transfer of isolates to new substrate plates is needed to ensure viability, however a less labor intensive method for long term storage has been developed. *A. apis* does not have asexual reproduction as most other insect pathogenic fungi. The infective units of *A. apis* are ascospores which are produced in round fruiting bodies. Formation of fruiting bodies and thus production of infective spores can be accomplished *in vitro* readily by the use of isolates of different mating types. The quality of newly produced spores should be assessed by a spore germination test before being applied. *A. apis* spores are activated by the presence of a high carbon dioxide level thus it is important for the spore germination test to be conducted in an environment with more than 10 percentage carbon dioxide. Detailed protocols are planed to be made available for the COLOSS community through the BeeBook and could serve as standards for future research on chalkbrood and honeybees.

Insecticidal maize seed coatings and honeybees- precautionary measures and experience from Austria 2009 and 2010

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The Austrian Federal Office of Food Safety set compulsory precautionary measures to be followed by seed plants and farmers concerning insecticidal maize and oil pumpkin seed coatings in order to reduce the exposure risk of honey bees.
In 2009 suspected bee poisoning incidents connected with corn sowing were reported from 28 apiaries. In these cases no total colony losses occurred, but losses of forager bees, house bees and bee brood and in some cases reduction of honey yield were observed. Samples of the affected apiaries were tested for residues of Clothianidin, Thiamethoxam, Imidacloprid, Fipronil and Fipronilsulfon. 83 % (n=29) of the bee samples and 64 % (n=36) of the bee bread samples showed positive results for at least one of the analytes. No residues were found in extracted spring honey samples (n=8). All plant samples (n=14) collected from off-crop areas near affected apiaries were tested positive for residues. Bee samples of the affected apiaries were analysed for parasites and pathogens, just in some cases they showed low infestation. As results indicate, the observed symptoms were linked with the period of maize sowing and the use of insecticidal maize seed coating.
Experience of the ongoing season 2010 will be reported.

Winter food usage economy: a key factor for reducing winter losses of bee colonies in long winter conditions

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Long winter in North Europe sets heavy requirements for overwintering bee colonies so as to survive until next season. Optimally colonies should overwinter with high overwintering index (colony strength in spring vs autumn) but there is also another aspect in the everwintering success. A large proportion of winter losses in Finland is caused by starvation. It is not always clear what makes some of similarly strong colonies to consume significantly more winter food than other colonies in same apiary. This might be caused, at least partially, genetically. The COLOSS GEI setup provided a template to compare winter food consumption of 4 bee strains in Finland. Differences in food consumption have also suggested to be influenced by methods used in varroa control, for example oxalic acid. As most of GEI colonies (n= 29) were not treated with oxalic acid and other colonies (n=33) were available that were treated with OA trickling treatment it was also possible to compare whether OA treatments increase the risk of losing colonies by starvation. No statistical differences in winter food consumption between groups LigF, CarL and CarB could be found, but MacGR consumed significantly (P=0,03) more than others. For example in comparison with FinL the consumptions were 17,37±3,30 and 13,99±2,94 kg. On the other hand, no statistical differences between OA –treated and nontreated colonies were found.

Bee colony losses in Slovenia in the year 2009/2010

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This year Slovene beekeepers experienced high honey bee colony losses which are similar to the losses in the year 2007/2008. The survey included more than 500 beekeepers and showed an average colony loss of 23% with the range from 0 to 100%. Losses differed among regions and were highest in the Costal - Karst area (41%). Most beekeepers (63%) noticed empty colonies without bees and 93% claimed to have enough food in the lost colonies. Differences in the loss could not be correlated with the type of medical treatments against the parasitic mite *Varroa destructor*, but with the number and time of treatments. The survey showed lower survival of bee colonies in the close vicinity of intensive agriculture indicating negative impact of agriculture on bee colony survival. Bee survival was higher at the higher altitudes compared to the lower altitudes which could be a result of lower transfer of diseases due to low density of bee colonies and impact of climate in the relation to slower varroa mite development due to longer brood free period and the abundant pasture clear of chemicals.

Colony losses in Sweden 2009-2010

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From 29th of April to 30th of May we have conducted a web based survey on colony losses in Sweden 2009-2010. 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 weeks of June and results presented at the meeting in Ankara.

Colony questionnaire in Spain

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In order to know the situation about colony losses in Spain in 2009-2010, COLOSS questionnaire has been spread out in different ways:

- Beekeeping meetings (Feria Apícola de Pastrana, March 2010)
- Beekeeping associations
- Veterinary services (Associations and Officials)
- Active survey currently being developed (more than 1000 beekeepers)

This COLOSS questionnaire is being coordinated by CAR (Centro Apícola Regional). Up to know we have got more than 100 questionnaires filled out but they are still going in. During the next month we have expectations of some hundred more.

Assessing genetic variability of honey bee origins used in the GEI experiment

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Different approaches to confirm the origin of honey bees are currently in use in Europe. However, only limited collections of accessible reference data are available. Therefore, a combination of generally employed methods will be used to analyze samples of the colonies that are part of the common GEI experiment of working group4 within COLOSS, for their geographic and genetic origin: morphometric analysis, mtDNA analysis, microsatellites and isozyme analysis. These data will be useful both for the documentation of the genetic origin of each colony involved in the common experiment, but also help to create a published and accessible reference database for honey bee diversity in Europe. Such a database appears of essential importance as an inventory of the currently available knowledge and as a prerequisite of defining gaps and further research needs, given that locally adapted bees may be better able to cope with diseases, parasites and environmental stress factors.

Selection of honey bee against the colony losses in Serbia

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Taking into account a number of causes that lead to a greater or less colony losses of honey bees, selection has proved to be the safest way to repair the disappearance of bees in Serbia. Honey bee selection is performed in five Centers with along eight different lines. After two years of trackings morphometric and productive characteristics, from the best bee colony takes the material for the forming of new line in the next two-year cycle of research and so on.
Across the winter of 2009/2010 years in Centers with the years-long selection were vanished 3-5% of bee societies. However, in Serbia in the larger and smaller apiaries, regardless of the type of hives, were losses 6.11-25.35%. By analyzing the data, the largest bee mortality was recorded in central and northern part of Serbia (20.37-25.35%) and lowest in the eastern part of Serbia where was dominant hives with selected queens.
Based on the results from previous years, with great certainty we can conclude that by honey bee selection, we selected such honey bee queens whose colonies was showed: greatest the productivity and purities of race, the greatest tolerance on diseases and the total loss in the our apiaries.

Co-infection of *Nosema cerenae* with Sacbrood and Chalkbrood infections in collapsed colonies of Turkey

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First molecular detections of Sacbrood virus, *Ascosphaera apis* and *Nosema cerenae* were achieved in Turkey at 2008 and 2009. Most of the samples were collected from collapsed colonies of national wintering areas.
At the beginning of the 2010 spring, remarkable clinical symptoms were observed and photographed by local veterinary doctors at different migrator apiaries deployed near a forested sea side after complaining of beekeepers. Sample collection and laboratory investigations applied according to OIE procedures and scientific literature. These symptomatic colonies were found infected all three topic infectious agents as co-infection in the same apiaries.
Impact of these records may be understand with scientific synthesis of molecular investigations and clinical symptoms of regional colony collapse cases.

Honey bee mortality in Belgium : a multifactorial study

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Since 2004, a multifactorial study was set up in order to discover the factors of risks which are likely to be linked with the high level of honey bee colony mortality in Belgium. A list of pesticides including both product types used in apiculture and in the surrounding crop fields has been set up and quantification of residues were carried out. The food availability in the environment and more specifically the quality and the essential amino acid contents of pollen were analysed. The pathologies were studied combining field observations, microscopic, classic microbiological and molecular approaches. Finally, the correlation between the colony mortality and the presence of pesticides, pathologies and the environment were analyzed. Eighteen pesticides were detected in the hives and two amino acids (isoleucine and tryptophan) were deficient in pollen but no relationship between these factors and the honeybee mortality was observed. Concerning the pathologies, the mite *Varroa destructor*, the American foulbrood at the clinical stage and the Acute Bee Paralysis virus were linked with increased colony mortality. Co-infection with more than two viruses before the overwintering also had an appreciable negative effect on colony survivorship.

Colony Losses of Honeybees in Turkey

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In Turkey, Beekeeping industry has a big potential for Turkish economy. Between 2006-2007, there were some problems with colonies. Coincident with Colony Collapse Disorder (CCD) news in the US, extraordinary colony losses have been reported in early 2007 from several eastern provinces of Turkey. In 2007, we have conducted a survey study on a subsample of beekeepers from around Turkey 288 questionnaries representing over 35.000 colonies. This analysis indicated an overall higher colony loss 30% When it comes to 2008, the important development for Turkish Beekeeping is revision of Beekeeper Registration System. Turkish Agriculture Ministry and Turkish Beekeepers Assocciation begin to use barcode system for registration from hive to honey jar. All data can be used for bee health monitoring system. Moreover, we can follow migrotary beekeeper and spread of diseases by means of this system. So, we registered 33.770 beekeepers' last situations of their 3.300.000 colonies. We applied simple questionnaire to determine colony losses especially winter losses in all regions of Turkey. In 2010, The basic Coloss Questionnariae was applied in Turkey to 1884 beekeepers. All data were analysed statistically and the colony closes rate was found 13,3% in 2010 spring time. When it is compared with the last two years, colony losses level has become higher. Besides, many Turkish Beekeepers claimed about weather conditions and viral symptoms of colonies during the period. Only 329 questionnariae could be translated to English and enter the data to Coloss Network for joint publication. After the second survey of questionnaire, all results would be more clear for Turkey.

The preliminary study about the antimicrobial activity of organosilicon quaternary ammonium chloride on American Foulbrood pathogen: *Paenibacillus larvae*

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The hydrolysis product of a quaternary amine-containing organosilicon salt (Si-QAC), 3-(trimethoxysilyl)-propyldimethyloctadecyl ammonium chloride exhibited antimicrobial activity against a broad range of microorganisms while chemically bonded to a variety of surfaces. In this study, the chemical was tested for American Foulbrood (AFB) pathogen: *Paenibacillus larvae.* AFB is a serious disease in honey bees (Apis mellifera). It is very common among the colonies in Turkey. So, AFB is also big problem economically in Turkish Beekeeping Industry. Si-QAC was examined for 28 different local strains of *Paenibacillus larvae* spore, vegatative and spore-vegatative forms and P. larvae strain ATCC 9545 Si-QAC was prepared by Nanotechnology Company, Istanbul. All strains were growth in Brain-Heart Broth Medium (Sigma, 42gr/L) and then transferred 0.1 ml bacteria ($1x10^8$ CFU/ml) MYPG Medium. The experiment was set up for spore and vegetative forms in 4 parts: 1.Inoculation of P.larvae spores/vegatative forms to MYPG medium added Si-QAC before, 2.Addition of Si-QAC to the medium and after drying process inoculation of P.larvae spores/vegatative forms, 3.Inoculation of P.larvae spores/vegatative forms to MYPG medium sprayed Si-QAC before, 4. Spraying of Si-QAC to the medium and after drying process inoculation of P.larvae spores/vegatative forms.
The results show that Si-QAC inhibits the bacterial growth significally. It has also bactesicid activity on spore form of P. larvae. The experiment will be extended by cage experiment for toxicity tests on honey bees.

Concerning vitality of honey bees in Bulgaria: Nosema situation

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A new investigation on diversity and vitality of honey bees in Bulgaria was realized in 2009 – 2010 period. The main purpose of the study was to characterize the following: 1) genetic variability of honey bees in different bio-geographical regions in Bulgaria and 2) "nosema" situation in the country. Totally 98 locations (apiaries) from all over the country were studied for presence of *Nosema ceranae* and *Nosema apis* species. A PCR technique was used for variability detection. All samples collection was during the spring-summer period of 2009. Five colonies per location were tested. *N. ceranae* have been found in 43 of the apiaries studied, which were about 44%. The *N.apis* has been detected as a present species only in two of apiaries – about 2 % of all studied. Different locations from border regions of Bulgaria (near to Turkey, Greece, Macedonia, Serbia and Romania) were also studied and *N. ceranae* detected in all of them. The *N. ceranae* invasion is characterized as a new for Bulgaria and its presence in the country is considered as a possible reason for increasing of colony losses in 2010.

Chemical products in Bosnia and Herzegovina beekeeping and Colony Losses

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Honey bee diseases and pests are just one issue on global causality on colony losses. Different countries have different legislation for *ad.us.vet* drugs in beekeeping practice. The other used group chemicals on market are free accessible which are also used. Treatments efficacy on pathogens can be measured trough their impact on honey bee health and consequently on annual losses. It will be interesting to compare global losses connected with drug availability and national drug registration standard.
Two years monitoring program on honey bee losses and data connection with additional, optional answers, on specific point of interest about *chemicals* that beekeepers using in practice, were combined and we receiving interesting data based on more than 5% BiH beekeepers in trial.
Loses during 2009/2009 and 2009/2010 where not so bad but beekeeping practice going on chemical treatments and drug National registration regulation were *not enough in a harmony*.
The usual practice in treatment of bee diseases and bee pests are not also in a harmony with
(EC) 2377/90 and MRLs for EU market can bee problem. Or if we see things differently maybe we can say that there are need (or maybe not) for less strict regulation on MRLs in the era of massive colony losses.

The colony losses in 2009/2010 in China

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1 Beijing , China;
2 Jilin, China;
3 Zhejiang, China;
4 Guangdong, China;
5 Hubei, China;
6 Liaoning, China;
7 Jiangsu, China

To evaluating the colony losses in 2009/2010 in China, COLOSS questioner was co-conducted with a Chinese project "Evaluating the negetive empact in main honey production regions of China" in 8 provinces of China (Gansu, Shanxi, Guangdong, Hubei, Jilin, Liaoning, Jiangsu, Zhejiang). In total, there are about 160 apiaries and 9,000 colonies were involved.
Apis mellifera and *Apis Cerana* are both employed in bee products production in Gansu, Shanxi and Guangdong province, therefore, the investigation was conducted both *Apis* species in these areas. For Hubei, Jilin, Liaoning, Jiangsu and Zhejiang province, only *Apis mellifera* colonies was explored. COLOSS questioners was send to the local organization of each province, in which the Chinese project is acting, and beekeepers were requested to filling the COLOSS questioner during the annual gathering meeting in their region. Data is under analysis.

Evaluation of colony losses in Israel: timing, presence of bee pathogens and varroa

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We are studying colony losses in Israel since 2008 aiming to characterize their distribution and extent and to evaluate their correlation with the presence of bee pathogens, pests and management practices. In this project we directly address the beekeepers through a survey using a pre-elaborated questionnaire and perform systematic monitoring of selected hives along the country. The questionnaire is distributed among the registered growers once a year. Ten sites (about 100 hives) were monitored along the year 2009. In 2010, two surveys are planed using a COLOSS questionnaire: for winter loses in May and for total losses in November. Five sites (20-30 hives each) are currently monitored.
In 2008 and 2009, 58 and 55 responding beekeepers, respectively, indicated that overall colony losses were below 20%. Hive-monitoring for the presence of pathogens revealed that although most of the hives appeared healthy, about 95% of them carried pathogens. The frequency of infections fluctuated along the year. Over all, the most common pathogens were *Nosema ceranae*, Black Queen cell virus, Israeli acute paralysis virus, Deformed wing virus and *Varroa Destructor* Virus. Also, recurrent seasonal outbreaks of Chronic bee paralysis virus and Acute bee paralysis virus were observed. Multiple pathogen infections were common.

Evaluation of pollen substitute by measuring total protein content of the honeybee (*Apis mellifera*)

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The lack of pollen is considered to be an important contributing factor to the so-called CCD

Supplementary protein diets assembled especially for bees have not been available in Hungary. This is why beekeepers used to apply different animal feeds (of calves etc.) often containing inadequate ingredients. We have been testing natural supplementary bee feed from 2008. According to the documentation supplied by the feed is composed by natural meal of different cereals not containing nor pollen or soy been or proteins of animal origin. In 2009 we continued testing a supplementary protein diet. For laboratory tests groups of newly hatched bees (at the same age) were put in incubator. Mixed pollen-, bee bread in sugar syrup and supplementary patty was given to bees with sugar candy controls. On the 12th day the bees were anaesthetized by CO_2 and stored deep frozen (-70 °C) until analyzed for body protein content. To measure total protein of the bee's body the Bradford (Coomassi-blue) protein assay was set to work, using BSA standard. Analysis was carried out in microtiter plates in automate ELISA reader.

Heads of the bees of different groups were also measured for comparing hypopharingeal gland development during the experimental period.

Field test of natural supplementary diet for bees

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The lack of pollen is considered to be an important contributing factor to the so-called CCD. We have been testing natural supplementary bee feed from 2008 on colony development, honey production, summer splitting of colonies, Nosema infestation, Varroa populations and general health conditions.
The early nosema testing in March revealed high spore counts in almost every colony. Two weeks later, much lower infestation was registered in the protein supplemented colonies. Surprisingly, spore counts dropped in all colonies according to the tests of May. However nosema infestation relapsed in the controls, to the end of summer, though out of supplementary patty consuming colonies only one showed spore shedding. Pollen and nectar shortage characterized the climate in summer of 2009. Against the fact no remarkable health problem, infectious or parasite disease was observed. The experimental splits consumed 700-750 g supplement syrup during the developmental period, the controls got sugar syrup only. The population data showed that experimental splits exhibited 21 % bigger population size and 35 % more brood than the control ones. According to our experience favorable protein supply from supplementary feeding seems to prevent bees from different negative stress as parasite infestations in nosemosis and maybe in varroosis.

Monitoring of winter honeybee colony losses in Poland

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In Poland we have performed a survey on winter colony losses since 2008, but with the use of the COLOSS questionnaire since 2009. The questionnaire was disseminated: during beekeepers' meetings and conferences, by email (or fax) messages and letters sent to beekeeping associations and individual beekeepers, through the most popular Internet fora for beekeepers, through the most popular Polish beekeeping journal "Pszczelarstwo". The survey was also performed on the website www. beemonitoring.org (courtesy of Romee van der Zee). However each year the participation of beekeepers was unsatisfactory (from 0.8 to 1%).

During the winter of 2007/2008 colony losses in Poland were about 15.3% and were higher than during the winter of 2006/2007 and 2008/2009 when they reached about 11 and 12% respectively. This year, until May 29, we received questionnaires from about 0.5% of Polish beekeepers. An analysis of these partial data showed that the colony losses last winter were about 17%, however in Wielkopolskie the losses were about 13%, while during the previous years they were one of the highest in Poland and reached about 30 % during the winter of 2007/2008 and 18%. during the winter of 2008/2009. Beekeepers attributed their losses to varroosis (20%), weak colonies in autumn (14%), nosemosis (13 %), poor queens (12%) and starvation (9%).

Winter losses of honey bee colonies in Republic of Macedonia (up date 2009/2010)

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Based on designed common COLOSS Basic Questionnaire for the colony losses 2009-10, the translated version was sent to the local beekeeping association in the country. We have received fulfilled 139 questioners back from the same number of beekeepers representing 1,39% of the total number of the beekeepers in Macedonia. They are located in 12 different regions across the country. The total number of the colonies of the assessed beekeepers was 7.763 which presents 7,1% of the total population of honey bee colonies in the country.
From the analyzed questionnaires, we can conclude that the average colonies' losses during the winter 2009/2010 was 6,7%, which is lower than the losses level from the 2007/2008 winter season. There are various detected reasons from assessed beekeepers such as: Varroa mite (7,9%), lack of food (7.9%), weak colonies in autumn (10,1%) and queen quality (11,5%).

D. List of participants

Participants' list

	Name-Surname	Country
1.	Benjamin Dainat	Switzerland
2.	Mića Mladenović	Serbia
3.	Bach Kim Nguyen	Belgium
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5.	Franco Mutinelli	Italy
6.	Almudena Cepero Rodríguez	Spain
7.	Kalinka Gurgulova	Bulgaria
8.	Petrov Plamen	Bulgaria
9.	Ulrike Hartmann	Switzerland
10.	Seppo Korpela	Finland
11.	Claudia Dussaubat Arriagada	France
12.	Malgorzata Bienkowska	Poland
13.	Maria Bouga	Greece
14.	Cecilia Costa	Italy
15.	Cristina Botias Talamantes	Spain
16.	Fani Hatjina	Greece
17.	Marina Meixner	Germany
18.	Selwyn Wilkins	UK
19.	Anna Gajda	Poland
20.	Victoria Soroker	Israel
21.	Nor Chejanovsky	Israel
22.	Karl Crailsheim	Austria
23.	Ljubiša Stanisavljević	Serbia
24.	Mustafa Necati Muz	Turkey
25.	Annette Schuermann	Germany
26.	Annette Bruun Jensen	Denmark
27.	Eva Forsgren	Sweden
28.	Elke Genersch	Germany
29.	Fancy Rojas	Chile
30.	Marie-Pierre Chauzat	France
31.	Julien Vallon	France
32.	Jean-Daniel Charriére	Switzerland
33.	Sreten Andonov	Former Yugoslavian Republic of Macedonia
34.	Norman L. Carreck	UK
35.	Fabrice Allier	France
36.	Violeta Santrac	Bosnia and Herzegovina
37.	Zlatko Tomljanovic	Croatia
38.	Svjetlana (Lana) Vojvodic	Denmark
39.	Ralph Schmidgall	Switzerland
40.	Dennis vanEngelsdorp	USA
41.	Per Kryger	Denmark
42.	Panuwan Chantawannakul	Tailand
43.	Jozef J. M. Van de Steen	Netherlands

44.	Ribière-Chabert Magali	France
45.	Aykut Kence	Turkey
46.	Meral Kence	Turkey
47.	Mohammad Forsi	Iran
48.	Beata Panasiuk	Poland
49.	Hannelie Human	South Africa
50.	Bjørn Dahle	Norway
51.	Hemma Kögleberger	Austria
52.	Yves Le Conte	France
53.	Ales Gregorc	Slovenia
54.	Vincent Dietemann	Switzerland
55.	Evgeniya Ivanova	Bulgaria
56.	Peter Neumann	Switzerland
57.	Raquel Martín-Hernandez	Spain
58.	Wei Shi	China
59.	Jasna Kralj	Slovenia
60.	Aleksandar Uzunov	Former Yugoslavian Republic of Macedonia
61.	Nikola Kezic	Croatia
62.	Joachim de Miranda	Sweden
63.	Romée van der Zee	Netherlands
64.	Preben Kristiansen	Sweden
65.	Hasan Hüseyin Ünal	Turkey
66.	Eyal Ben-Chanoch	USA
67.	Gal Yarden	Israel
68.	Aslı Elif Sunay	Turkey
69.	Emre Yıldırım	Turkey
70.	Robert Varon	Turkey
71.	Mehmet Binboğa	Turkey
72.	Kamyar Ahmadi	Iran
73.	Cristian Pirk	South Africa
74.	Giovanni Formato	Italy
75.	Aslı Özkırım	Turkey
76.	Aygün Yalçınkaya	Turkey