Action FA0803

Proceedings of the

5th COLOSS Conference

Prevention of honeybee COlony LOSSes

In the Montpellier SupAgro

2, Place Pierre Viala 34060

Montpellier, France

September 14-15, 2009

Dear colleagues

On behalf of the organising team, I would like to welcome you to the 5th COLOSS conference at the University of Montpellier.

I would like to thank 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. Maria Navajas and her team.

Appreciation is also addressed to all contributors for submitting their abstracts, which I hope will stimulate rewarding discussions on colony losses and the underlying factors and mechanisms. Please keep in mind that the focus of this meeting will be to plan our activity until the next COLOSS conference in Ankara, Turkey.

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.

Peter Neumann, Action Chair

Bern, Switzerland, Wednesday, 19 August 2009

The Local Organizing Committee for the 5th COLOSS Conference

Fabrice Alier, Vincent Girod, **Jean-Francois Martin**, **Maria Navajas**, Magali Ruello, Bernard Vaissiere

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Agenda

Time	Programme
13th September 2009 (Sunday)	
19:00 -	EC Meeting (first meeting point : Hotel Ibis, Montpellier-95, place Vauban Boulevard d'Antigone - 34000 - MONTPELLIER - FRANCE Tel : (+33)4/67640664 Fax : (+33)4/67659173)
14th September 2009 (Monday)	
08:00 – 09:00	Registration
09:00 – 09:30	General Assembly JF Martin – M. Navajas – P. Neumann: Welcome and plenary session: organizational and COST matters
9:30 – 9:45	Plenary talk by J Pettis
9:45 – 10:00	Plenary talk by JD Ellis
10:00 – 10:15	Plenary talk by RFA Moritz
10:15 – 10:30	Plenary talk by W. Meikle
10:30-11:00	*Coffee break with snacks*
11:00 – 11:15	Plenary talk by R. Büchler
11:15 – 11:45	Work shop reports (5-10 min each) by respective organizers R. van der Zee (Amsterdam), P. Neumann (Bern), N. Kezic (Unije)
12:00 – 13:30	Separate WG meetings: detailed plans 2009-2010
13:30 – 14:30	*Lunch*
14:30 – 16:00	WG mix: WG leaders together with colleagues, who have this WG as a 2nd choice
16:00 – 16:30	*Coffee break with snacks*
16:30 – 18:30	Separate WG meetings and discussion of results from the WG mix
18:30 – 19:30	Poster session
20:00-	*Social dinner*
15th September 2009 (Tuesday)	
09:00 – 10:30	Plenary session – final discussions, planning of the next meeting
10:30 - 11.00	Coffee break with snacks
11:00 – 13:00	MC Meeting of the COST Action FA0803

Registration on site is required.

Registration fees: 10 €

CONFERENCE LOCATIONS
Montpellier SupAgro **(http://www.supagro.fr/)** **2,Place Pierre Viala 34060 Montpellier, France** **(Access map: http://www.supagro.fr/web/pages/open_plan_acces.html)**

CONTACT DETAILS OF LOCAL ORGANISERS	
Maria Navajas	**Jean-Francois Martin**
Address: Centre de Biologie et Gestion des Populations (CBGP) Montpellier SupAgro Campus International de Baillarguet CS 30 016 34 988 Montferrier sur Lez Cedex **Phone :** 00 33 (0)4 99 62 (33 20) / (33 34) **GSM:** 00 33 (0)75 99 45 73 *(only in case of extreme emergency)* **e-mail**: navajas@supagro.inra.fr>	**Address:** Centre de Biologie et Gestion des Populations (CBGP) Montpellier SupAgro Campus International de Baillarguet CS 30 016 34 988 Montferrier sur Lez Cedex **Phone :** 00 33 (0)4 99 62 (33 20) / (33 34) **GSM:** 00 33 (0)613 588 573 *(only in case of extreme emergency)* **e-mail:** martinjf@supagro.inra.fr

CONTACTS FOR INFORMATIONS	
Dr. Asl› Özk›r m	**Dr. Fani Hatjina**
Hacettepe University Dep. of Biology 06800 Beytepe/ANKARA/TURKEY Phone: 00 90 312 297 80 43 Fax: 00 90 312 299 20 28 **e-mail:** ozkirim@hacettepe.edu.tr	Hellenic Institute of Apiculture (N.AG.RE.F.) 63 200 N. Moudania, Greece Tel: +30 23730 91297 Fax: +30 23730 91676 **e-mail:** fhatjina@instmelissocomias.gr

Summary of Contributions

By Asl› Özk›r m & Fani Hatjina

Contributions for the Vth Coloss Meeting in Montpellier include 56 different abstracts from 26 participating countries. Data for Colony losses is reported in 12 of the above abstracts. The new data (presented in bold) has been added into the table prepared by Crailshem *et al*. after the IVth Coloss meeting in Zagreb. In this way we can always monitor the changes occurred in each country (Table 1). Most countries did not report any new data but highlighted the need for detailed survey.

The rest of the abstracts are referred to other aspects related with colony losses and they were classified under 9 subjects/ topics, in order to obtain standardized and easy-read information out of them. In this summary (Table 2), you can find your subject of interest and see all other abstract titles related to the same subject (including authors' names and proceedings' page number for each abstract).

From Table 2 it is obvious that there is no abstract concerning losses of honey bees during pollination process, although pollination is the major service provided by honey bees to the ecosystem. Broadening our research field will provide us with a better understanding of Colony Losses.

Table 1. Updated Colony Losses

Country	Beekeepers	Colonies	Years	Losses(%)	N=sample size	Type of Survey	Susceptible Region(s)	N.cereane	IAPV	Page(s)
Austria	22198	278.810	2006/2007 2007/2008 **2008/2009**	- 13.3 **9.7**	- 16.217 **14.299**	- Questionnarie **Questionnarie**	**Western Regions/Carinthia**	YES	NO	22
Belgium	8600	101.600	2006/2007 2007/2008 **2008/2009**	7.3 - **-**		Questionnarie, visit, sampling		nd	nd	
Bosnia and Herzegovinia	-	-	2006/2007 2007/2008 **2008/2009**	- - **-**				nd	nd	
Bulgaria	40000	750.000	2006/2007 2007/2008 **2008/2009**	6 10 **5**	13.000 13.000 -	Phone survey Phone survey **Phone survey**		nd	nd	23
Croatia	-	-	2006/2007 2007/2008 **2008/2009**	- 27 **13.16**	- 10.293 **35.108**	Questionnarie **Questionnarie**		YES	nd	25
Czech Republic	-	-	2006/2007 2007/2008 **2008/2009**					nd	nd	
Denmark	4100	-	2006/2007 2007/2008 **2008/2009**	- 32 **-**	- 17.000 **-**	Questionnarie		YES	NO	
Finland	2700	53.000	2006/2007 2007/2008 **2008/2009**	- 15.5 **-**	- 3.514 **-**	- Voluntary survey **-**		YES	NO	
Former Yugoslav Republic of	10000	75.000	2006/2007 2007/2008 **2008/2009**	- 18 **-**	- 11.912 **-**	- Questionnarie **-**		NO	NO	

Macedonia										
France	69000	1.300.000	2006/2007 2007/2008 **2008/2009**	- 29.3 -	- 62.400 -	CNDA survey	**North-EastFrance**	YES	YES	26
Germany	85000	900.000	2006/2007 2007/2008 **2008/2009**	8-16 8-16 -	7.200 7.200 -	DEBIMO DEBIMO		YES	YES	
Greece	22000	1.300.000	2006/2007 2007/2008 **2008/2009**	15 14 -	26.000 48.250 -	Questionnarie Questionnarie -		YES	nd	
Hungary	15000	800.000	2006/2007 2007/2008 **2008/2009**	- 10-30 -	- 170 -	- Diagnosticprogram -		YES	NO	
Ireland	2000	20.000	2006/2007 2007/2008 **2008/2009**	53 15-20 -	891 - -	Questionnarie Unofficalestimates **Questionnarie**		YES	nd	27
Israel	450	100.000	2006/2007 2007/2008 **2008/2009**	- - **40**	- - **46.000**	- - **Questionnarie**		YES	YES	28
Italy	75000	1.157.133	2006/2007 2007/2008 **2008/2009**	- 37.4 **13/24**	- 5.973 **10.940/12.360**	- Questionnarie **Questionnarie/survey**	**Emilia-Romagna**	YES	NO	29
Netherlands	8000	-	2006/2007 2007/2008 **2008/2009**	- 23 -	- 7.434 -	- NCB Dutch Monitor -		YES	NO	
Norway	3000	60.000	2006/2007 2007/2008 **2008/2009**	- 10.1 -	- 17.872 -	- Questionnarie -		YES	NO	
Poland	40000	1.000.000	2006/2007 2007/2008 **2008/2009**	- 15.3 **8**	- 26.710 -	- Questionnarie **ColossQuestionnarie**		YES	nd	30
Portugal	15000	550.000	2006/2007	30.3		National survey				

			2007/2008 **2008/2009**	- **-**		- **-**		nd	nd	
Serbia	20000	400.000	2006/2007 2007/2008 **2008/2009**	- 27.5 **-**		- Estimation **-**		YES	NO	
Slovenia	8000	170.000	2006/2007 2007/2008 **2008/2009**	- 30-50 **-**		- Estimation **-**		YES	nd	
Spain	24606	2.464.601	2006/2007 2007/2008 **2008/2009**	14-89 - **-**	1.957 - **-**	Survey - **-**		YES	YES	
Sweden	12000	125.000	2006/2007 2007/2008 **2008/2009**	12 17 **17.5**	33.800 31.400 **7.354**	Questionnarie Questionnarie **Questionnarie**		YES	nd	31
Switzerland	18000	190.000	2006/2007 2007/2008 **2008/2009**	- 18 **-**	- 8.200 **-**	- Monitoring system **-**				32
Turkey	200000	3.850.000	2006/2007 2007/2008 **2008/2009**	30 1.8 **-**	35.000 1.250.000 **-**	Survey Questionnarie **-**		YES	nd	
United Kingdom	41000	290.000	2006/2007 2007/2008 **2008/2009**	- 33 **-**	- 10.897 **-**	- Questionnarie **-**		YES	NO	
USA	-	2.400.000	2006/2007 2007/2008 **2008/2009**	31 36 **28.6**	- - **-**	Apiary Inspectors America/USDA-ARS **Apiary Inspectors America/USDA-ARS**		YES	YES	
China	-	-	**2008/2009**	**A.mellifera 5** **A.cerena 30**				nd	nd	24

Table 2. Subject Classifications

SUBJECTS	
1. Varroa and Varroa Control	
- The enemy of my enemy: Treating bees with entomopathogenic fungi against varroa mites	*William G. MEIKLE*[1 2], Guy MERCADIER[1], Marie-Claude BON[1], Fatiha GUERMACHE[1], Vincent GIROD[3], and Niels HOLST[4]* *p.19*
- Winter Survival of Honeybee Colonies Depends on the Timing of Varroa Control	*Tjeerd Blacquière*, Bram Cornelissen, Lonne Gerritsen and Jozef van der Steen* *p. 34*
- Ants and mites: where is the link?	*Benjamin Dainat[1*], Rolf Kuhn[1] , Daniel Cherix[2] , Peter Neumann[1]* *p.38*
- Small cells against Varroa: filling the gaps	*Vincent Dietemann** *p.39*
- The influence of the drones to the varroa tolerability	*Marica Maja Dražić[2], Lidija Svečnjak [1], Dragan Bubalo [1], Janja Filipi [3], Gordana Hegić [1], Ivan Mihaljević [1], Nikola Kezić [1]* *p.40*
- Oxalic acid: Toxicology on Apis mellifera	*Eva Rademacher* and Marika Harz* *p.61*
2. Pesticides	
-Role of Temperature Related to Bee Age in the Response to Pesticides	*Gherardo Bogo[1,*], Piotr Medrzycki[1], Simone Tosi[1], Laura Bortolotti[1], Fabio Sgolastra[2]* *p.35*
- Role of food quality in bee response to pesticides	*Simone Tosi[1,*], Piotr Medrzycki[1], Gherardo Bogo[1], Francesca Grillenzoni[1], Laura Bortolotti[1], Fabio Sgolastra[2].* *p.65*
3. Genetic Diversity	
- Colony Losses and Genetic Diversity of Honey Bees in Greece	*Maria Bouga[1]*, Fani Hatjina[2], Leonidas Charistos[2]* *p.36*
- Genetic variation in honeybee populations:a valuable resource at the time of global bee losses	*Meral Kence* , Rahsan IvginTunca* *p.47*
- Genetic diversity of honey bees (Apis mellifera L.) in Turkey revealed by RAPD markers	*Tunca, R.I.[1,2]*, Koleoglu, G.[1], Atagan, Y.[1], Kence, M.[1]* *p.66*
- Diversity of Honey Bees (Apis mellifera L.) On The Territory of Republic of Macedonia	*Aleksandar Uzunov[*], Hrisula Kiprijanovska and Sreten Andonov* *p.67*

Plenary Lectures

European test on genotype - environment interactions

Ralph Büchler[*1], *Ina Heidinger*[1], *Marina Meixner*[1], Malgorzata Bienkowska[2], Beata Panasiuk[2], *Yves Le Conte*[3], *Didier Crauser*[3], *Cecilia Costa*[4], *Fani Hatjina*[5], *Meral Kence*[6], *Nikola Kezic*[7], *Seppo Korpela*[8], *Per Kryger*[9], *Antonio Murilhas*[10], *Aleksandar Uzunov*[11], *Jerzy Wilde*[12]

[1]LLH, Bee institute, Erlenstrasse 9, 35274 Kirchhain, Germany
[2]Research Institute Research Institute of Pomology and Floriculture, Apiculture Division, 24-100 Pulawy, Poland
[3]INRA, UMR 406 Abeilles et Environnement, Laboratoire Biologie et Protection de l'abeille, Site Agroparc, Domaine Saint-Paul, 84914 Avignon Cedex 9, France
[4]CRA-API, Bee and Silkworm Research Unit, Via di Saliceto 80, 40128 Bologna, Italy
[5]Hellenic Institute of Apiculture (N.AG.RE.F.), N. Moudania, Greece
[6]Middle East Technical University, Biology, 06531 Ankara, Turkey
[7]Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia
[8]MTT, Agrifood research Finland, 31600 Jokioinen, Finland
[9]University of Aarhus, DJF, Research Centre Flakkebjerg, 4200 Slagelse, Denmark
[10]Universidade de Evora, ICAAM, 7000 Evora, Portugal
[11]Faculty for Agricultural Science and Food, bul. Aleksandar Makedonski b.b., 1000 Skopje, Republic of Macedonia
[12]Apiculture Division, Warmia and Mazury University, Sloneczna 48, 10-710 Olsztyn, POLAND

* Author for correspondence: ralph.buechler@llh.hessen.de

Observations about local strains of bees that are apparently less affected by losses and have better strategies to cope with Varroa have been reported from different regions. It is a major goal of the Coloss network to better understand the impact of genetic differences and of genotype-environment interactions on colony losses.

As an activity of working group 4, a common protocol is being developed to test for the vitality of European honeybees, and a comparative survival experiment has been started in July 2009. At 16 different test locations all over Europe, 18 different strains and ecotypes of honeybees will be evaluated. The parameters recorded will include the population dynamics of bees and brood, the honey/food balance, the occurrence and development of bee diseases (Varroa, Nosema, viruses etc.), and the hygienic behavior.

The constant and close monitoring of the colonies will allow the development of threshold values for infestation rates with Varroa mites and their implementation into a treatment regime.The key observation parameter will be the survival period of untreated colonies.

The results will contribute to our understanding of the interactions within the system bee-mite-environment. Additionally, we expect to use the results for the development of sustainable management strategies adapted to different regions of Europe.The results will constitute a significant contribution to one of the key objectives of working group 4: the development of internationally recognized criteria of vitality and the compilation of standardized methods to assess colonies using these criteria, resulting in methodological and technical recommendations for breeders.

CCD Research in the United States

*James D. Ellis**

Honey Bee Research and Extension Laboratory, Department of Entomology and Nematology, University of Florida, P.O. Box 110620, Bldg. 970 Natural Area Dr., Gainesville, FL, 32611-0620

*Author for correspondance:jdellis@ufl.edu +352 273 3924

It has been difficult to assess the impact of Colony Collapse Disorder (CCD) in the U.S. The Apiary Inspectors of America (AIA) and USDA-ARS estimate that honey bee colony losses for fall/winter 2006-2007 and 2007-2008 were 31% and 36% respectively. Data collected by the AIA and USDA-ARS in March 2009 indicate a 28.6% colony loss rate during the 2008-2009 fall/winter. Although the rate of colony losses decreased in 2008-2009, the cause(s) of CCD in U.S. bee colonies remains under investigation. To that end, there are number of research efforts in the U.S. attempting to address the cause(s) and control of CCD. These efforts largely are divided into two principle areas of investigation: intra-colony stressors and extra-colony stressors. Regarding the former, researchers in the U.S. focus primarily on the potential role of bee pests/pathogens, management stress, queen source, bee nutritional fitness, and chemical use in bee colonies in eliciting/exacerbating CCD-like symptoms. Regarding extra-colony stressors, researchers consider chemical toxins in the environment, poor forage availability, climatic stress, etc. on the role of CCD. Considerable headway has been made in understanding the role many of these play in general colony health although no clear cause of CCD has been discovered. Some of the highlights of CCD research in the U.S. include, in no particular order:

1) the discovery of Israeli Acute Paralysis Virus and its presence in colonies expressing CCD-like symptoms,
2) the discovery and genome mapping of *Nosema ceranae*,
3) the presence of pesticides in bee colonies and how pesticides affect adult and immature bees lethally and sublethally,
4) the role nutrition plays in immune system stress,
5) how varroa and associated pathogens promote colony demise,
6) the development and economic analyses of new methods of pest/disease control,
7) determining how management stress affects colony productivity,
8) and how multiple stressors interact to promote colony loss.

Although the cause(s) of CCD has not been discovered, U.S. researchers, with their international colleagues, have made significant advances toward understanding bee health in general. As scientific collaborations continue to develop, U.S. researchers welcome/invite collaboration with international partners.

The enemy of my enemy: Treating bees with entomopathogenic fungi against varroa mites

William G. MEIKLE[1 2], Guy MERCADIER[1], Marie-Claude BON[1], Fatiha GUERMACHE[1], Vincent GIROD[3], and Niels HOLST [4]*

[1] European Biological Control Laboratory, USDA – ARS, Campus International de Baillarguet, CS 90013 Montferrier sur Lez, 34988 St. Gely du Fesc, France.

[2] current address: Honey Bee Research Unit, Kika de la Garza Subtropical Agricultural Research Center, 2413 E. Highway 83, Weslaco, TX 78596

[3] ADAPRO LR-CRALR, Maison des Agriculteurs, Mas de Saporta CS 30012, 34875 Lattes, France.

[4] Danish Institute of Agricultural Sciences, Flakkebjerg, 4200 Slagelse, Denmark.

*Author for correspondance

Strains of an entomopathogenic fungus, *Beauveria bassiana*, were isolated from varroa found in beehives in France and were tested in lab bioassays. Fungal conidia were formulated with plant-based wax powder and used to treat hives in 5 independent field experiments. The main variables measured over time were: 1) mite fall onto sticky boards; 2) phoretic mite density; 3) mite infection rate; and 4) number of fungal conidia per bee. Colony health was monitored in terms of total colony weight, brood surface area, adult population weight and food reserves. In experiments with 1 to 3 consecutive applications of biopesticide (separated by 7-10 days), mite fall was significantly affected and repeated applications resulted in significantly lower mite fall. Mite infection rates declined faster in the spring than in the fall. The number of conidia per bee declined from about 10^4 conidia per bee to fewer than 10 per bee in 7-14 days. Treatment did not affect hive survivorship or amount of brood. However, phoretic mite densities were not significantly reduced. In an experiment to evaluate 4 consecutive biopesticide applications, more than half the treated hives died. The biopesticide was found to be contaminated with *Pseudomonas fluorescens*, a common bacterial contaminant of stored food and wastewater. Laboratory experiments with mixed cultures showed that *P. fluorescens* interfered with B. bassiana growth. The bacteria were found in two shipments of commercially-produced conidia but not in laboratory-produced conidia. We encourage other researchers to check for bacterial contamination in all biopesticide research.

Is breeding for disease resistance a sustainable concept in honeybees?

*Robin Moritz**

Institut für Biologie Martin-Luther-Universität Halle-Wittenberg Hoher Weg 4 06099 Halle/Saale, Germany

*Author for correspondance:Robin.Moritz@zoologie.uni-halle.de

Breeding for disease resistance has become a major goal in selection programmes of the honeybee. Often these efforts comprise international consortia, the utilization of genetically broad and diverse stock as a basis for selection. Repeatedly, there have been reports of disease resistance stock and they are marketed at a global scale.

In spite of the repeated successes of breeding, the breeding results are disappointingly unsustainable. Resistant stock comes as quickly as it disappears. This may not be surprising given the structure of the apicultural industry. The complex mating biology of the honeybee queen is a notoriously complex handicap to implement resistant stock at the large scale. In light of the ever increasing suite of pests, parasites and pathogens it is even highly questionable whether it is a desirable selection goal to spread resistant stock at a large scale. Regional selection programs, aiming to maintain diversity may be the only feasible way to develop a breeding strategy for disease resistance. Even than we should not expect to reach a sustainable disease resistant breeding line, it will require constant breeding efforts to keep ahead of colony losses due to pathogens of the honeybee.

Honey bee colony losses in the United States

*Jeff Pettis**

USDA-ARS Beltsville, MD USA and Dennis vanEngelsdorp, Pennsylvania Department of Agriculture, USA.

* Author for correspondance: Jeff.Pettis@ARS.USDA.GOV

About one third of all overwintering honey bee colonies in the U.S. have been lost in each of the last three winters. A survey conducted by the Apiary Inspectors of America USDA-ARS surveyed beekeepers in an attempt to quantify the extent of bee losses in the U.S. Honey bee colony losses in 2007, 2008, and 2009. Losses were reported to be 31% in the winter of 2007 (vanEngelsdorp et al. Amer. Bee J. 147:599-603, 2007) 36% in the winter of 2008, (vanEngelsdorp et al. PLOSone 2009) and 29% in the winter of 2009 (unpublished).

The recent 07-08 survey did not find a significant difference in the size of the beekeeping operation (<50 colonies, 50-500 colonies, or >500 colonies) and colony losses. Additionally, there was no relationship between total colony loss and whether the beekeeper moved between multiple states and or to California for almond pollination. The only significant finding with size or type of beekeeping operation was that larger operations reported more often CCD-like symptoms. A longitudinal study that examined pests and pathogen levels in three commercial beekeeping operations over the past year will be reported on. Additionally, the full results of these surveys, including new data on colony losses from the 2008-2009 survey and recent pathogen levels within dead and dying colonies will be presented and discussed.

PART-A

Austria

Colony losses in Austria, a 2 years survey

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

[1]Institute for Zoology, Karl-Franzens University Graz, Universitätsplatz 2, A-8010 Graz, Austria
[2]Austrian Agency for Health and Food Safety, Institute for Apiculture, Spargelfeld-straße 191, A-1220 Vienna, Austria

* Author for correspondence: Karl.Crailsheim@uni-graz.at

To survey overwinter losses of honeybee colonies we handed out a questionnaire at 8 beekeeping conventions in Austria in 2008. In 2009 we conducted a mixed media survey (attending conventions and distributing the questionnaire online and through a beekeeping journal). We obtained information on more than 5% of Austrian honeybee colonies in both years. In 2007/08 total loss was 13.3% (2158 of 16217 colonies, 374 operations), ranging from 9.2% in Salzburg to 17.1% in Lower Austria (including Vienna). In 2008/09 total loss in Austria was 9.7% (1391 of 14299 colonies, 454 operations), ranging from 4.4% in Upper Austria to 22.1% in Carinthia. Losses were highest in the mountainous western regions (Salzburg, Tyrol, Vorarlberg: 16.6%) and Carinthia. In both years, beekeepers attributed their losses mainly to invertebrate pests (*Varroa destructor*), queen loss, starvation and hive management. We also asked beekeepers about their hive management and found no effect of date of last feeding and type of food fed before winter on median colony mortality ($p>0.05$, Kruskal Wallis test). Furthermore, median overwinter mortality did not differ between operations that used an essential oil (thymol) and organic acids or organic acids only. We also did not find differences in overwinter mortality of small (1-50 colonies) and large (50+ colonies) operations ($p>0.05$, Kruskal Wallis test). In 2008/09 we found that migratory beekeeping resulted in lower total loss (5.1%, 48 operations) than stationary beekeeping (10.7%, 380 operations, $chi^2=118.8$, $p<0.05$). In 2009 we conducted a mixed media survey where we obtained questionnaires online, from a beekeeping journal and at beekeeping conventions. The median overwinter mortalities of individual regions did not differ between these different ways of data collection ($p>0.05$, Kruskal Wallis test).

The degree of overwinter mortality of honeybee colonies in Austria in the last two years is stable and – with few regional exceptions – on a low level. We attribute the degree of mortality to well known causes, with *Varroa destructor* presumably playing a key role.

Bulgaria

Bulgarian honeybee colony losses during 2008/2009

Evgeniya Neshova Ivanova[1]*, *Plamen Pavlov Petrov*[2]

[1]University of Plovdiv “Paisii Hilendarski”

[2] Agricultural University – Plovdiv

*Author for correspondance: geneiv@uni-plovdiv.bg

In recent years, beekeeping in Bulgaria has expanded because of the diversity of local flora and the possibilities for production of different types of honey. At present, there are about 750 000 bee colonies which are object of professional and amateur beekeeping in Bulgaria. The structure of the sector indicates that beekeeping in Bulgaria still has an extensive and scattered character (50% of beekeepers have a small number of bee colonies, 41% have up to 50 colonies). The health status of the bee colonies in Bulgaria is under the control of the National Veterinary Service and its regional structures. The bee colonies are controlled for *Varroa destructor*, *Acarapis woodi*, *Nosema apis*, *Bacillus alvei*, *Bac. laterosporus* (Bac. Orpheus), *Melissococcus pluton* (Streptococcus pluton), *Enterococcus faecalis* (Streptococcus apis) and *Peanibacillus larvae* once per year. Till the moment, Bulgaria is free from *Acarapis woodi* and IAPV. According to unpublished data from a new research supported by Bulgarian Agricultural Ministry, *Nosema cerana* is present in the country*.* According to a phone survey with members of Bulgarian Beekeeper’s Union and National Bee Breeding Association from all over the country, the colony losses in Bulgaria during the period 2008/2009 are about 5% on the average. This percent is less than 2007/2008 when about 10% colony losses were reported. The survey represents 5% of the bee colonies (from small to medium scale beekeepers) but all of the geographic areas in the country.

Data about the percentage of colony losses in different regions of the country is as follows: North-west – 2%; North-central – 3-5%; North-east – 10-12%; South-east – 2%; South-central – 3%; South-west – 3%. Technological reasons (winter nutrient stores), *Varroa* and *Nosema*, conduction of plant protection, disinfection and disinsection activities are possible reasons for bee colony losses in Bulgaria during this period.

China PR

Colony losses in China in 2009

Shi Wei

Apicultural Research Institute, Chinese Academy of Agricultural Sciences

Xiang Shan Beijing 100093, China

In 2009, the investigation of colony losses on both spices of honeybee, (*Apis mellifera* and Apis *Cerana*) has made in 10 main apiculture provinces of China. Data were collected both by the local beekeeping organizations and the bee institute of Chinese Academy of Agricultural Sciences. The results indicated that the average of colony losses during the over-wintering period of *Apis mellifera* were 5%. However, a big looses were occurred in spring of 2009 on *Apis cerana* colonies over the country, in average there were about 30% colonies were deeded, and in some areas there were up to 80% colonies were loss.

Croatia

Possible reasons for colony losses in Croatia

Zlatko Tomljanovic[1]*, Ivana Tlak Gajger*[2]

[1] Croatian Agricultural Extension Institute
Fra Andrije Kacica Miosica 9/III, 10000 Zagreb, Croatia

[2] Department for Biology and Pathology of Fish and Bees, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia

There are approximately 300.000 honey bee colonies and 12.000 beekeepers in Croatia, but just 4132 beekeepers are members of Croatian beekeepers federation (CBF). They produce around 6500 – 7000 tons of honey annually. Croatian beekeepers federation estimate (based on questionnaire) that honey bee colony losses for 2005/2006 and 2007/2008 were 16% and 27% respectively. In 2008 347 beekeepers (with and without losses) were interviewed about their losses. We have collected data concerning: 1) migratory/stationary beekeeping operations, 2) bee yard site, 3) size of beekeeping operations /number of colonies/, 4) treatment against Varroa mite – when? medication?, 5) type of microclimate and landscape, 6) honey flow conditions /late summer/, 7) beekeeping practice, and 8) first clinical signs of disappearing of bees. We suppose that higher losses for fall/winter 2007/2008 are mainly due to: 1) bee pests and pathogens, 2) pesticides, 3) weather,and 4) bad beekeeping practice. During the June 2008 CBF sent to each beekeeper a guide for Varroa control. We agree that there is a lack of well-trained and experienced beekeepers for helping in commercial queen breeding and in all beekeeping industry in southern Europe. Queen breeders and beekeepers from other parts of Europe, USA and Australia claim the same thing. Five years ago CBF have started program “School for beekeepers” certified by Ministry of science and technology of Republic of Croatia. The purpose of program is permanent education of beekeepers that are beginners and also well experienced beekeepers. Program includes 120 hours of theoretical and practical lectures. Beekeepers study about bee biology, queen rearing, bee health, beekeeping economy, beekeeping technology and equipment, pollination and bee flora and apitherapy. There are more than 2500 certified beekeepers so far. Likewise, CBF organizes a fall-winter scheme of lectures for their members with domestic and foreign speakers. In the survey about colony losses in Croatia 2008/2009; 10, 40% of CBF members were included. The data was collected directly from the beekeepers during the annual beekeeping meeting at the end of March 2009. In total, 430 beekeepers responded to an anonymous questionnaire and a survey was run on 35 108 bee colonies that exhibited 13.16% of winter losses. CBF will continue with data collection and support further research concerning Varroa fitness.

France

Estimation of honeybee colony losses within professionnal beekeepers in France during winter 2007/2008

F. Allier, L. Bournez, A. De Boyer, V. Britten, P. Jourdan, I leoncini, J Vallon*

CNDA Centre National de Développement Apicole, 149, rue de Bercy – F-75595 PARIS Cedex 12.

*Author for correspondance:fabrice.allier@cnda.asso.fr

In recent years, professional french beekeepers have found an increase in winter honeybee colony losses (mortality, weakness, diseased or queennless). At the moment in France, neither monitoring nor survey was released in order to assess bee losses. That is why the Health Committee of the CNDA (Centre National du Développement Apicole) has undertaken an investigation into this issue during the winter 2007-2008 with a sampling of professional beekeepers.
168 professional french beekeepers (more than 150 hives) were randomly selected out of 782 beekeeping farms. Therefore, this survey includes 1358 apiaries and 62400 colonies. A questionnaire was sent to beekeepers. An average of 29.3% (IC95% = [26% - 32%]) of losses was recorded, ranging from 21 to 62%.

The beekeepers estimated the mortality rates of colony during the winter 2005-2006 and 2006-2007 being 16.8% and 17.3 % respectively. Some regions (North-East of France) were more affected than others.

Dead colonies represented 50% of the losses, when queenless and diseased colonies were 14% and 8% respectively. The rest (28%) was weak colonies.

Preliminary results for possible causes show that availability of food, strength of the colonies and varroa pressure could explain partly the losses.

A further analysis will give us results about the correlation between bee colony losses and the different variables studied, in which we find beekeeping practices for wintering preparation (varroa treatment), colony background during the season, environment of apiary and colony poisoning due to pesticides (acute poisoning or depopulation).

We intend to extend this national survey over several years to get a close monitoring of loss rate. We'll be able, next September 2009, to show the first figures on bee colony losses during winter 2008/2009.

Ireland

Monitoring colony losses in Ireland

Mary F Coffey[1,*] *John Breen*[2]

[1]University of Limerick, Dept of Life Sciences, Ireland.
[2]University of Limerick, Dept of Life Sciences, Ireland.

*Author for correspondence: Mary.Frances.Coffey@ul.ie

In Ireland, beekeeping is primarily practiced as a hobby and it is estimated that approximately 2000 beekeepers manage 20 000 colonies. Prior to the introduction *Varroa desructor* in 1998, expected winter losses was approximately 10%, however post varroa reported colony losses are 15-20%. The perceived cause of colony losses amongst beekeepers is poor mating of queens during the Autumn and inadequate control of *Varroa destuctor* and its associated viruses. However, colony losses may alternatively be caused by single or multi-infestation with other endemic pathogens such as tracheal mite, AFB, EFB and *Nosema apis*. *Nosema ceranae* was detected in Ireland for the first time in 2008, but its frequency of occurrence has not been documented. In 2006, a preliminary assessment of the incidence of disease in colonies was carried out by sampling adult bees and brood from a total of 136 colonies randomly selected through out the country. Presently, these samples are been analyzed for the presence or absence of tracheal mite *Nosema apis*, *Nosema ceranae* and *Varroa destructor.* Incidence of DWV, CBPV, IAPV and KBV is also been assessed. However, with the exception of the above survey, most of data on bee diseases and colony losses in Ireland is based on informal and subjective beekeepers assessments, thus could not be compared with data from other countries or be used with reliability to develop disease control strategies which would minimize colony losses. Therefore, this Autumn we have planned to carry out the first formal assessment of colony losses using the COLOSS working group 1 questionnaire. This will be implemented in close collaboration with the University of Limerick and the Federation of Irish Beekeeping Association. To reduce costs, it is hoped that the survey will be disseminated and coordinated primarily by the secretaries in each of the 46 associations distributed around the country and registered and non-registered beekeepers will be encouraged to complete the survey. In localities where this method of dissemination is not feasible, mail and email will be used. To identify any problems with misinterpretation of questions by beekeepers in the proposed questionnaire, a trial run was carried out during May 2009 on losses incurred by members in 5 associations (*n*=70 beekeepers) during 2008/2009. Small problems which were identified will be addressed before disseminating the final questionnaire

Israel

Progressing Survey of Honeybee Colony losses in Israel

Chejanovsky N. [1], Hetzroni A[2], Yacobson B[3], Voet H[4], Slabezki Y[5], Efrat H[5] and Soroker V[1]**

[1]Department of Entomology; Institute of Plant Protection
[2]Department of Sensing, Information and Mechanization Engineering Agricultural Research Organization, the Volcani Center, 50250 POB 6 Bet Dagan, Israel
[3]The Kimron Veterinary Institute
[4]Faculty of Agriculture, Food and Environment The Hebrew University of Jerusalem
[5]Extension Service Ministry of Agriculture

*Authors for correspondence:ninar@volcani.agri.gov.il, sorokerv@volcani.agri.gov.il

Recent reports on colony losses worldwide and local reports of about 25% of beehive decline motivated us to initiate a comprehensive study of the incidence and characteristics of colony losses in Israel. To evaluate symptoms and extent of colony decline and losses and the respective roles of pathogens, parasites and pesticides and management practices we are carrying:

1. Survey of honeybee colony losses and its potential causes via mail, phone and email; 2. Systematic sampling of healthy and problematic beehives after requeening, in the fall; at the end of winter before adding suppers; and after honey harvest in the summer. 3. Detection of pathogens including viruses and *Nosema ceranae*, by sensitive RT-PCR and microbiological means. Dedicated computerized tools are implemented for data collection and analysis.

From about 46,000 colonies (58 beekeepers) for 2008, about 40% complained for extensive colony loses. Some of them observed classical CCD symptoms. In 2009, from 113 hives that were directly examined and sampled for pests and pathogens towards the end of the winter, 18.6 % of hives showed *Nosema cerana* and 19.4 % *Varroa destructor*. Analysis for viruses revealed the presence of Acute Bee Paralysis virus (ABPV), Black Queen Cell virus (BQCV), Chronic Paralysis Bee Virus (CBPV), Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV), Sacbrood virus (SBV) and Varroa derived virus 1 (VDV-1). IAPV, DWV and BQCV were detected most frequently. Kashmir Bee virus (KBV) was not detected. A significant negative correlation was found between workers population in the hive and the presence of viral infection.

Italy

Colony losses in the Italian region Emilia-Romagna during summer 2008 and winter 2009

*Giacomo Vaccari, Cecilia Costa, Marco Lodesani**

CRA-Unità di ricerca di apicoltura e bachicoltura, Bologna, Italy

*Author for correspondance: marco.lodesani@entecra.it

The reports of losses from Italian beekeepers in recent years indicate that these are highest in spring (possibly caused by incorrect use or abuse of pesticides) and during the winter (probable cause infestation by the ectoparasitic mite *Varroa destructor* and associated viral diseases).

Emilia-Romagna is one of the Italian regions with the highest number of honey bee colonies (~ 100,000 colonies, corresponding to ~ 10 % of the total national number). To obtain an overview of colony losses in this region during spring-summer 2008 and during winter 2008-2009 two surveys were carried out by means of distribution of anonymous questionnaires.

For summer losses (losses which occurred between spring and autumn 2008) data from 119 beekeepers, managing 10,940 colonies, was collected. In this survey summer losses of 1,443 (13%) colonies were reported. In April 2009 data were collected from 199 beekeepers managing 12,360 colonies in autumn 2008, who reported that 2,930 colonies (24%) were dead in spring 2009. In the questionnaires, 108 beekeepers expressed an opinion on the cause of death: 36% attributed it to varroa, 18% to multiple infestations, 9% to nosema, 9% to lack of honey stores, 4% to poisoning by agrochemicals, and 3% to American foulbrood.

Norway

Honeybee Colony Losses in Norway 2008-2009

*Bjørn Dahle**

Norwegian Beekeepers Association
Dyrskuev. 20 NO-2040 Kløfta Norway

*Author for correspondance: bjorn.dahle@norbi.no

In Norway honeybee colony losses are surveyed annually in fall. A questionnaire is sent to all the members of the Norwegian Beekeepers Association, representing more than 90 % of the beekeepers in Norway. Some 12-20 % of the beekeepers return the questionnaire. Data from this survey for calculation of the losses during winter 2008-2009 is thus not available yet. A smaller survey among 30 beekeepers that are partners in the national breeding program for honeybees and that are distributed throughout the county was carried out this summer. The results suggest that winter losses are not higher than 10%, which has been the average rate of colony losses during the last 10 years. Further, beekeepers are asked to report extraordinary colony losses, but no such reports have been received for the winter 2008-2009. Waiting for the data from the annual survey my conclusion is so far that colony losses during the winter 2008-2009 were within the normal range.

Poland

Monitoring of winter (2008/2009) honey bee colony losses in Poland

Grażyna Topolska, Anna Gajda*

Warsaw University of Life Sciences, Faculty of Veterinary Medicine,
Ciszewskiego 8, 02-786 Warsaw, Poland

* Author for correspondence: grazyna_topolska@sggw.pl +48225936140

During the winter of 2007/2008 colony losses in Poland were about 15.3%. The data on bee losses were gathered using questionnaires distributed among beekeepers, mainly during various meetings and conferences. 431 (1%) Polish beekeepers with 26710 (3%) bee colonies participated in the survey. After the winter of 2008/2009 an Internet survey was introduced. It was conducted through the www. bee.monitoring.org website, using the Coloss questionnaire. Information about the monitoring was disseminated: during beekeepers meetings and conferences, by email (or fax) messages sent to beekeeping associations, through the most popular Internet fora for beekeepers, through announcements in two beekeeping journals. It was also possible to fill in the hard-copy questionnaire at some of the beekeepers' meetings. Until the beginning of June the response of the beekeepers was low. The reasons for this will be discussed in the presentation, together with the results of the analysis of all the gathered data. The analysis of the data gathered until now suggests that general colony losses during the winter of 2008/2009 were lower than during the previous winter and reached about 8%. Both Internet and hard-copy surveys suggest that losses in smaller apiaries (with up to 50 colonies) were higher than in bigger apiaries. The owners of bigger apiaries constituted a higher percentage of respondents in the Internet survey than in the hard-copy survey.

Sweeden

Colony losses in Sweden – latest data

Preben Kristiansen[*1]

Swedish Beekeepers Association, Trumpetarev 5, SE-59019 Mantorp, Sweden

*Author for correspondance:preben.kristiansen.sbr@biodlarna.org +46 735 233 122

The number of beekeepers and colonies in Sweden is about 12000 and 125000 respectively. The Swedish Beekeepers Association (SBR) has since many years received reports from its members about the number of colonies and honey production etc. Swedish data on winterlosses are based on theses reports, and the average losses for each 10 years period between 1975 and 2005 were 10-12 %, varying from year to year between 6 and 22 %.
The losses 2006/2007 and 2007/2008 were 12 % (n= 33800) and 17 % (n= 31400) respectively. These figures are based on reports from 4296 and 3714 beekeepers respectively (which is about 44 % and 41 % of the members of SBR). Information on colony losses 2008/2009 based on the yearly reports to SBR, will not be available until March 2010.
In order to get a quicker overview of the colonies that were lost during the fall and winter of 2008/2009 we conducted an internet based survey. A link to the questionnaire was published on the website of SBR on the 8th of May and the survey was closed the 5th of June. Information about the survey was sent by e-mail to all the local and county beekeepers clubs, which have an e-mailadress. In total 307 persons received information by mail.
The questionnaire included the following questions on colony numbers: number of colonies in 2008, number of wintered colonies, number of lost colonies, number of colonies by the 1st of May and number of weak colonies. It even included questions on whether Varroa was present in the colonies and on Varroa control methods. It was voluntary to fill in name and address, but compulsory to fill in zip code and e-mailadress. It was however clearly stated in the questionnaire that all data would be anonymised.
We received 565 answers. Some of them represent more than one beekeeper, so we don't know the exact number of beekeepers that the survey represents, but it is about 6,5 % of the members of SBR and about 5 % of the estimated number of beekeepers in the country.
The number of colonies that were wintered in 2008 was 7354 (about 6% of the estimated number of colonies in Sweden). By the 1st of May 6084 of those colonies were alive, which means that 17,5 % of the wintered colonies were lost.
In the group of colonies where Varroa is still not present, the mortality rate was 10,3 % (n=652). Whereas it was 18,1 % in the group where Varroa is present (n=6702). About 50 colonies with Varroa were not treated in 2008, the mortality rate in that group was 42,3 %.

Switzerland

Colony losses in Switzerland: newest results

*Jean-Daniel Charrière**

Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, Schwarzenburgstrasse 161, CH-3003 Bern, Switzerland

*Author for correspondance: jean-daniel.charriere@alp.admin.ch +41 31 323 82 02

In Winter 2007/08, we start a close collaboration between the Swiss Bee Research Centre and the beekeeping associations. A representative panel of 472 beekeepers (2.6% of the CH-beekeepers), distributed overall Switzerland and managing 8200 colonies, are invited via e-mail to provide data about their winter losses on a website. We got so the first results from the newly established national monitoring system which was implemented. From the 1st of October 2007 till the 1st of April 2008, the mean colony losses in Switzerland were 18% (ranging from 5-35% depending on the canton).

For the winter 2008/09 we use the new developed COLOSS basic questionnaire. Unfortunately, do to logistical and personal problems, the panel was contacted only in June and the responding rate was reduced in comparison with the former years. So, for the winter 2008/09 we collected data on 331 apiaries distributed overall Switzerland. The participants to the questionnaire announced a total of 5038 wintered colonies. Till the spring (1st of April 2009) 8.8% of these colonies died and 8.1% were too weak in spring to develop to a production colony. Among the 443 colonies which died during the winter, 72% disappeared with none or only a few living bees remaining, while enough food supply was present and 60% had patches of capped brood. In general, we can say that the colony losses were reduced during the last winter even so some few beekeepers observed high colony mortality on their apiaries. The goal for the next spring is to contact the panel via e-mail already at the beginning of April to increase the response rate and to find new participants to the panel. We aim at 5% of the Swiss beekeepers participating to the questionnaire.

Until now, the major suspect in Switzerland is clearly V. destructor in close association with other pathogens like viruses or bacteria. Nosema ceranae has been confirmed in Switzerland but is not correlated with higher mortality. We can't exclude that pesticides used in agriculture or in apiculture are also involved.

PART-B

Winter Survival of Honeybee Colonies Depends on the Timing of Varroa Control

Tjeerd Blacquière, Bram Cornelissen, Lonne Gerritsen and Jozef van der Steen*

Plant Research International, Wageningen University & Research, 6700 AB, Wageningen,The Netherlands

*Author for correspondance:tjeerd.blacquiere@wur.nl

Infestation by varroa mites of a cell with a pupa causes the developing bee to have a shorter life expectancy, which may be especially crucial in case of winter bees. In two consecutive experiments the effect of the timing of varroa control treatments (July, August, September or December) on the life span of individual bees and the survival of colonies during winter was examined.

Results:

- most winter bees hatched in September and October
- life span in late treated colonies was less than in early treated
- early treated colonies showed less infestation before and during hatching of winter bees
- late treated colonies had prolonged brood rearing in autumn
- many of the late treated colonies died during winter, no losses were recorded when colonies were early treated
- differences between season 2005-06 and 2006-07 reflected different climatic conditions

Role of Temperature Related to Bee Age in the Response to Pesticides

Gherardo Bogo[1,], Piotr Medrzycki[1], Simone Tosi[1], Laura Bortolotti[1], Fabio Sgolastra[2].*

[1] Agricultural Research Council, Research Unit for Beekeeping and Silk Breeding, Via di Saliceto 80, 40128 Bologna, Italy
[2] Department of Agroenvironmental Sciences and Technologies – Entomology, University of Bologna, Viale G. Fanin 42, 40127 Bologna, Italy

* Author for correspondence: gherardo1985@hotmail.com

Previous studies have evidenced that the pesticide effect on honey bees may depend on the temperature. Moreover it is known that all the individuals of a beehive may, directly or indirectly, enter in contact with pesticides.

The present research was aimed to study the response, in terms of mortality, of the adult bees of different ages (newly emerged, hive bees and foragers), following ingestion of different doses of Clothianidin, in relationship to the test temperature.

The newly emerged bees were obtained by isolating a queen bee for about 30 hours on a comb in a queen-excluding cage. In this way a high number of eggs were laid in a narrow interval of time allowing to obtain coetaneous brood. The queen was subsequently removed and the comb was left isolated, to avoid further egg deposition. The comb was kept inside the hive for 20 days to assure the most natural conditions. Subsequently it was moved to an emergence cage and kept at 34,5°C. Three days after beginning of the emergence, also hive bees and foraging bees were collected from the same beehive where the queen was previously isolated. Thus, the three groups of honey bees originated from the same queen.

The bees of each group were used to test LD50 of Clothianidin at three different temperatures: 20°C, 25°C and 35°C. For this purpose, six different doses of the active ingredient diluted in 50% sucrose syrup were provided to the groups of 20 individuals. After the complete consumption of the test solutions, the bees were maintained at the definite temperatures (i.e.: 20°C, 25°C and 35°C respectively) and 50% water solution of sucrose was provided *ad libitum*. Bee mortality was checked after 3, 6, 12, 24, 48 and 72 hours and LD50 of Clothianidin was calculated for each age group and each test temperature. In this way the influence of the test temperature on the response of honey bees of different ages to the administration of the a.i. was evidenced.

The discussion regards the possible effects of weather conditions on the susceptibility of different age honey bees to the intoxication by pesticides. The importance of bee age and test temperature for laboratory toxicity testing, was also discussed.

Colony Losses and Genetic Diversity of Honey Bees in Greece

Maria Bouga[1], Fani Hatjina[2], Leonidas Charistos[2]*

[1]Lab of Agricultural Zoology & Entomology, Agricultural University of Athens, 75 Iera Odos Str., Athens, 11855, Greece. Tel.: E-mail address:

[2]Hellenic Institute of Apiculture, N. Moudania,Greece

*Author for correspondance: mbouga@aua.gr ,+302105294575

It is possible that local strains of bees are less affected by losses and have better strategies to cope with Varroa or even other pathogens. Therefore the detection of local honey bee populations and the effort to persuade beekeepers to use them instead of foreign ones is very important

The s methodical study on genetic structure of honey bees has begun in Greece on 1994. The research is going on. The following approaches are used:

- Molecular markers (mitochondrial DNA: RFLP's and Sequencing).
- Isoenzymic analysis.
- Classical morphometrics analysis
- Geometrical morphometrics analysis.

The results of this research till now are:

- *A.m.macedonica* can be discriminated using RFLP's method from the rest populations existing in Greece, as well as from Cypriot, Italian, Turkish and Albanian honey bees.
- *A.m cypria* can be discriminated using isoenzymic analysis.
- *A.m ligustica* can be discriminated using RFLP's method from Greek honey bees and *A. m cypria*.
- Turkish, Bulgarian and Serbian honey bees can be discriminated using RFLP's method from Greek honey bees and Cypriot honey bees.
- Africanized honey bees can be discriminated using RFLP's method from Greek honey bees, as well as from Cypriot honey bees.

The above experience and the know-how will be used in order to certify the origin of queens which will be exchanged between groups and tested in different locations in the comparative survival test undertaken within members of WG4 (genotype and environment interactions experiment).

A case control study and a survey on mortalities of honey bee colonies (*Apis mellifera*) in France during the winter of 2005-2006

Chauzat Marie-Pierre, Zeggane Sarah, Drajnudel Patrick, Clément Marie-Claude, Martel Anne-Claire, Ribière Magali, Aubert Michel, Schurr Frank and Jean-Paul Faucon*

Agence Française de Sécurité Sanitaire des Aliments, Site de Sophia Antipolis, Unité Pathologie de l'Abeille, Les Templiers, 105, route des Chappes, BP 111, 06902 Sophia Antipolis, France

* Author for correspondance: mp.chauzat@afssa.fr + 33 (0)4 92 94 37 21

Several cases of mortality of honey bee colonies (varying from 38 to 100%) were observed in France during the winter of 2005-2006. In order to explain the causes of these mortalities, one case control was conducted in a limited area, and a larger survey was conducted in 18 other apiaries located in 13 sites over the national territory. Both studies included the diagnosis of the main honey bee diseases, the colony management measures taken by beekeepers and the research of pesticide residues in apicultural matrices. Pollen analysis was carried out on beebread samples to identify the floral species that were foraged before colony death. Poor *V. destructor* treatments together with nosema disease and brood diseases were frequent in apiaries with high colony mortalities. The results of the study designated the absence of any preventive treatment against V. destructor as the main risk factor. In the meantime, toxicological factors were not considered as the main explanation for the mortality of colonies. Results from other sites located throughout the national territory made it possible to extend the same conclusions to these other sites.

Ants and mites: where is the link?

Benjamin Dainat[1], Rolf Kuhn[1], Daniel Cherix[2], Peter Neumann[1]*

[1] Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP,
CH-3003 Bern, Switzerland
[2] Department of Ecology and Evolution, Université de Lausanne, CH-1015, Switzerland

* Author for correspondence: benjamin.dainat@alp.admin.ch

Ants are scavengers and often forage in honeybee, *Apis mellifera*, hives. However, their impact on quantitative diagnosis of the ectoparasitic mite Varroa destructor using natural mite fall has never been rigorously quantified. Here, we quantified the natural mortality of *V. destructor* using bottom board counts and excluded or allowed foraging ants in the respective hives. Our results clearly show that foraging by three different ant species has a highly significant impact on mite quantification. Since *V. destructor* is the major global pest for apiculture and an accurate mite quantification is crucial for pest management decisions, we herewith recommend appropriate ant control measures for both scientists and beekeepers.

Small cells against Varroa: filling the gaps

*Vincent Dietemann**

Swiss Bee Research Center, Agroscope Liebefeld-Posieux ALP, 161 Schwarzenburgstrasse, 3003 Bern, Switzerland

*Author for correspondance: vincent.dietemann@alp.admin.ch

Since the 90's some beekeepers rear their bees on combs made out of foundation with smaller cell imprints (4.9mm) compared to the usual foundation (5.4mm). This is based on the fact that foundation commercially produced for bees to build on generates cells of larger than those built naturally by honeybees. It is claimed that Varroa infestation is less severe when bees are reared in small, 'more natural' cells. The smaller space available for Varroa within the cell coupled with the shorter development time of individuals reared in small cells is hypothesised to reduce growth of the parasite population. Rearing bees on smaller cells thus represents an attractive natural alternative to using chemicals to control the parasite. Tests of this method are reviewed. Although this seems to be an easy question to answer, no conclusive demonstration has yet been obtained regarding the efficacy of the use of small cells to reduce Varroa infestations since evidence of both poor or good efficacy is reported. The factors that could influence the outcome of these tests are analysed. I discuss whether it is worth for beekeepers to invest the effort of 'retrogressing' their bees onto small cells or to use honeybee strains adapted to small cells.

The influence of the drones to the varroa tolerability

Marica Maja Dražić[2], Lidija Svečnjak [1], Dragan Bubalo [1], Janja Filipi [3], Gordana Hegić [1], Ivan Mihaljević [1], Nikola Kezić [1]

[1] Faculty of agriculture University of Zagreb, Svetošimunska 25 Zagreb, Croatia

[2] Croatian agricultural agency, Ilica 101, Zagreb, Croatia

[3] Polytechnic “Marko Marulic”, Knin, Croatia

*Author for correspondance:

Carnica (*Apis mellifera carnica*) breeder queens from German and Austrian selection programs regarding Varroa tolerance criteria were used as mother queens for drone colonies at the Unije Island mating station. Drone colonies were kept under moderate varroa pressure. Drone combs were introduced to the colonies to stimulate drone rearing.

An experimental apiary with 24 colonies was established during June 2008 in continental Croatia. All colonies have started with sister queens from a local Carnica breeder mated in differential locations. 12 queens were mated at the conventional mating station and 12 queens at the varroa tolerance mating station on the island Unije.

The goal of this experiment is to find out influence of pre-selected drones on tolerability traits of colonies in conventional conditions. Colony strength and varroa infestation were observed and measured at the experimental apiary.

During experimental period colonies with queens mated on the Island of Unije had higher colony strength. Average varroa infestation of colonies with queens from conventional mating was 3,65% with range from 1,36 to 11,92%, while at colonies with queens mated at the Island of Unije, average infestation was 4,43% with range from 1,50 to 10,57%. There were no statistically significant differences in the colony strength and infestation levels between tested colonies.

Most probable causes of colony losses during the winter of 2008/2009 in Poland

Anna Gajda, Grażyna Topolska*

Warsaw University of Life Sciences, Faculty of Veterinary Medicine, Ciszewskiego 8, 02-786 Warsaw, Poland

* Author for correspondence: anna_gajda@sggw.pl, +48225936140

In Poland, the winter of 2008/2009 brought considerably lower colony losses than the winter of 2007/2008. The aim of this study was to point out the most frequent causes of colony losses during the winter of 2008/2009. We examined 210 dead bee samples from collapsed or almost collapsed colonies. They were sent to the Warsaw University of Life Sciences (WULS) by 54 beekeepers between September 1, 2008 and May 7, 2009. The samples were examined for *Varroa destructor*, *Nosema* spp. and bees with deformed wings. We also performed an AGID test to check samples for the presence of acute bee paralysis virus (ABPV), black queen cell virus (BQCV) and chronic bee paralysis virus (CBPV). The comparison of gathered data showed that *Nosema* spp. infection was present in 79% of the samples. In 40% of the latter heavy infection by *Nosema ceranae* occurred. *Varroa* mites and infection by BQCV (detectable by AGID) were present in 5.7% and 8% of the samples respectively. In 10% of apiaries the probable cause of colony losses was *Varroa* infestation. In 60% of apiaries a severe *Nosema* spp. (often accompanied by BQCV infection) appears to have been the probable cause of colony collapse. In 87% of the latter *N. ceranae* was present. Bees with deformed wings were found in four apiaries and in individual apiaries infection by ABPV or CBPV (detectable by AGID) was present.

Honey bee colony losses in Turkey and their possible causes

Tuğrul Giray[1], Meral Kence[2], Devrim Oskay[3], Mehmet Ali Döke[2], Aykut Kence[2]*

[1] University of Puerto Rico, POB 23360, San Juan, PR, USA, 00931

[2] Middle East Technical University, Department of Biology, 06531, Ankara, TURKEY

[3] Washington State University, Pulman, WA, USA.

* Author for correspondence: aykut@metu.edu.tr, +90 312 2105176

Concurrent with emergence of Colony Collapse Disorder (CCD) in the US, we observed considerably very high colony losses early 2007 from several eastern provinces of Turkey. We have carried out a survey study on of beekeepers from around Turkey. There were very high regional losses with the average of 30% bee deaths. We investigated whether there is a significant correlation between extraordinary winter losses and the losses occurred previous years. We tried to pinpoint the possible causes of bee deaths by analyzing a battery of questions. We investigated several hypotheses: factors related to beekeeping locations, irregular season, known bee diseases, colony collapse disorder, honey bee genetic source, use of different beekeeping inputs such as sugar feed, wax foundation, queens, and parasite and disease treatments. Through recursive partitioning analysis we determined that the two most important factors are the geographic location of the beekeeper and the over-wintering location used. We found that bee genetics or race could only be secondarily important. We continue our survey study on colony losses in Turkey.

Evaluation of the virulence of deformed wing virus (DWV) for honey bees

*Sebastian Gisder, Nadine Möckel, Elke Genersch**

Institute for Bee Research, Hohen Neuendorf, Germany

* Author for correspondence: elke.genersch@rz.hu-berlin.de

Managed honeybees (mainly *Apis mellifera*) are the economically most valuable pollinators of crop monocultures worldwide. In addition, honeybees play a vital role in pollinating wild flowers. Hence, honeybees are important for both, a profitable and sustainable agriculture and the conservation of many non-agricultural ecosystems. The decline of managed honeybees, therefore, is of increasing concern. It is often suggested that a variety of non-sustainable farming practices, including pesticide use, are responsible for an increase in frequency, magnitude and geographical distribution of honeybee colony losses caused by a complex of symptoms called CCD (colony collapse disorder). Another line of evidence suggests that diseases also play a role in this phenomenon. Sound data exist for seasonal colony losses in Spain proving that *Nosema ceranae* is threatening honeybee well-being in this region of Europe. Data from Germany imply that there it is the mite *Varroa destructor* and certain viral infections (ABPV and DWV mostly) which are responsible for a considerable proportion of winter losses. Especially DWV infections were significantly linked to winter mortality. Therefore, our main focus during the last years has been on the virulence of DWV. We will present our data on vertical, horizontal and vectorial transmission of DWV and discuss the implication these data have for the evaluation of DWV virulence.

The neglected majority: common bacteria can induce mortality in adult honeybee workers

*Ulrike Hartmann******, *Alexandra Roetschi, Jean-Daniel Charrière, Peter Neumann*

Swiss Bee Research Centre, Agroscope Liebefeld-Posieux Research Station ALP, Schwarzenburgstrasse 161, CH-3003 Bern, Switzerland

* Author for correspondance:hartmann.ulrike@alp.admin.ch +41 31 324 74 24

Oral infections with bacteria can cause mortality of adult honeybee workers but virulence has only been tested for few species. Here we use *Pseudomonas aeruginosa* as a positive control and test *Morganella morganii* and *Lactobacillus kunkeei*. For 6 days, sugar solutions of the three bacteria (5×10^8 bacteria/ml) were fed *ad libitum* to adult workers in hoarding cages ($N = 6$ with 20 bees each). The controls ($N = 2$ cages) received only sugar solution and mortality of the workers was daily quantified. *Pseudomonas aeruginosa* induced the significantly highest mortality. Mortality due to *M. morganii* was significantly lower but higher than for *L. kunkeei* and the controls, where not a single dead worker was found. The data confirm that oral infections with *P. aeruginosa* can induce high mortality and further suggest that bacteria can significantly differ in their virulence. Since both *P. aeruginosa* and *M. morganii* are frequently found in honeybees and their hives, the role of bacteria other than *Melissococcus plutonius* and *Paenibacillus larvae* may have been underestimated in honeybee pathology.

Comparison between anatomical/ physical characteristics of queens and the performance of their sisters during the next productive period

Fani Hatjina[1], Asli Ozkirim*[2]*

[1] Hellenic Institute of Apiculture, N. Moudania, 63 200, Greece

[2] Hacettepe University, Department of Biology, Bee Health Laboratory, Beytepe-Ankara-Turkey

*Author for correspondance: fhatjina@instmelissocomias.gr +302373091297

The quality of the queen of a social insect such as the honey bee affects indisputably the colony's performance. High quality' queens tend to have the following physical characteristics: high weight, high numbers of ovarioles, large volume of spermatheca, high numbers of spermatozoa, no diseases. Beekeepers' demand for mated queens is great in a specific time of the year and for large numbers. Therefore, newly mated queens are sold very quickly after onset of oviposistion without allowing evaluation of their performance for a long period. It is thus essential that the evaluation of the quality of the queens is done in a quick and most precise way. The Laboratory for Verification of Honey Bee Queens' Quality of the Hellenic Institute of Apiculture (N.AG.RE.F.) meets the above demands by using a number of methods which can be apply to the specimens in stable laboratory conditions and give accurate and repeatable results. For the determination of the quality of young laying queens, the following parameters are measured by the above accredited laboratory: percentage of worker brood cells in an area of a 7.5cm diameter's circle (modification of Collins, 2000), percentage of empty brood cells in the same brood area, the number of ovarioles, the diameter of spermatheca and the presence of Nosema spores in queen's alimentary canal.

In order to test the relationship between the anatomical/ physical characteristics of Verified queens with the performance of their sisters during the next productive period, an empirical study started to take place in Spring - Summer 2009. A number of 30 colonies were allowed to produce their own queens, while reared and fertilized queens were introduced in a number of other 30 colonies. All 60 queens were evaluated according to the above criteria. Half of the queens from each group will be dissected while the rest will be allowed to perform freely and they will be evaluated for their performance during the following months.

Susceptibility of different honey bee subspecies: *Apis mellifera carnica*, *A. m. ligustica* and *A. m. mellifera* to chalkbrood, *Ascosphaera apis*.

*Annette Bruun Jensen**

Department of Ecology and Agriculture, University of Copenhagen,

Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

*Author for correspondance:

Chalkbrood is a brood disease caused by the fungus *Ascosphaera apis*. This disease rarely causes colony lose, but due to its common occurrence it may have a high impact on overall honey bee health status. In this study chalkbrood susceptibility of in vitro reared honey bee larvae was investigated. Larvae were grafted from 3–4 colonies headed by pure mated queens of *Apis mellifera carnica*, *A. m. ligustica* and *A. m.mellifera*, respectively. Three day old larvae were fed with different dosages of *A. apis* spores and a clear dose-response relationship was shown. LD50 estimates ranged from 55 to 905 spores. The dosis-response differed significantly (up to a factor ten) between colonies of the same subspecies. The mean time to death decreased with increased dosage; more larvae dying faster after eating more fungal spores. The *A. m. ligustica* larvae used in this study were less susceptible to chalkbrood than *A. m. mellifera* and *A. m. carnica* larvae. However due to the limited number of colonies tested and the high variation shown, we cannot predict that any *A. m. ligustica* colony in general is better adapted to cope with chalkbrood than colonies of *A. m. carnica* and *A. m. mellifera*.

Genetic variation in honeybee populations:a valuable resource at the time of global bee losses

Meral Kence , Rahsan IvginTunca*

Middle East Technical University, Biology Department, 06531 Ankara, Turkey

* Author for correspondence: mkence@metu.edu.tr, +90 312 2105169

Changes in environmental conditions can influence the survival of organisms and species. As the genetic diversity of a species declines, that species becomes increasingly at risk of extinction. It is stated that in honeybee (*Apis mellifera* L.) populations of US which is not native to this country there is a very little genetic variability and this condition was suggested as the most likely factor in recent Colony Losses. Genetic variation is considered as an insurance for the populations and species living in changing environments. Mitochondrial COI-COII region restriction analysis in honey bees of Turkey revealed C lineage haplotypes in Anatolia, carnica-type in part of Thrace, and A haplotypes in two southeastern locations where *A. m*. *syriaca* is distributed. The genetic diversity studied in honey bee populations in Turkey using six microsatellite markers revealed mean gene diversity levels ranged from 0.45 to 0.74. The number of private alleles and pairwise F_{ST} values support that the Anatolian honey bees have high level of genetic differentiation. When compared with the mean gene diversities per locus ranged between 0.23 and 0.40 for western European, 0.41 and 0.57 for northern Mediterranean, 0.76 and 0.90 for African populations, gene diversities in honey bees of Turkey were higher than that of the populations of Europe but lower than that of the tropical African populations. Investigations on the levels of bee deaths have shown differences between the bees of different genetic origins which may be affected by other behavioral characteristics. Genetic diversity in morphometrical, biochemical, and behavioral traits are of utmost importance in the face of global climatic changes and disease agents for adaptation and survival of the honey bees.

Nosema situation in Finland

*Seppo Korpela**

MTT Agrifood Research Finland, FIN-31600 Jokioinen, Finland

*Author for correspondance: seppo.korpela@mtt.fi +353 3 41882576

In Finland the winter with no flight possibility to bees extends often from mid-October up to mid-April, i.e. lasting even half of the year. The long winter can cause defecation into the hive and provoking the classical *Nosema apis* symptoms which contribute to the weakening and even losses of colonies. Since the discovery of *Nosema ceranae* in Finland (Paxton et. al 2007) I have asked beekeepers to send bee samples taken in spring from colonies that either show classical *Nosema* symptoms or depopulation symptoms that might be connected with *Nosema ceranae*. Also, I have established a network of 21 beekeepers in South Finland to monitor 1-2 apiaries (ca. 10 colonies) from which bee samples both in autumn and spring are collected. Within this group we will especially concentrate to find out whether *Nosema ceranae* is capable of causing more winter losses than *N. apis.* In autumn 2008 we inspected 208 samples. 32 had *Nosema* and in 6 samples the number of spores was higher than 10 million/bee with a maximum of 43,5 million/bee. Nosema species have been determined by PCR and the overall result was 8 samples with *N.a*., 12 with mix *N.a.+ N.c.* and 11 with *N.c*. The spore load tended to be higher with N.c. present, i.e. 1.5, 5.5 and 8.5 million spores/bee for the groups respectively. The spring samples were not yet checked for species as of writing, but some high values (highest was 218 million/bee) and colony losses already have been found in apiaries showing pure *N.c.* infestations in autumn. Examples of the losses will be presented.

In spring 2008 we checked 265 dead bee samples sent by voluntary beekeepers and *Nosema* was detected in 51,3% of the samples. From these, only 7% were pure N. apis. The general samples from spring 2009 have not yet been processed but we have at least one puzzling case to solve: whether it is the first detected massive colony loss in North Europe caused by *Nosema ceranae* or not. We detected a spore load of 48,2 million/bee in the 42 dead colonies in the overwintering apiary of one beekeeper. They had a total of few decilitres of bees left in the hives as compared to 30 alive cololonies in different overwintering apiary with only a 3,3 million/bee spore load and bees overwintering well.

Influence of sublethal doses of amidacloprid on break out of nosemosis in honey bees

*Jasna Kralj**

National Institute of Biology, Vecna pot 111, 1000 Ljubljana, Slovenia

*Author for correspondence: jasna.kralj@nib.si, +386 1 4233388

Amidacloprid is nicotinoid used for dress seeding to protect plants against insects and thus it presents hazard for honeybees. Several effects of sublethal doses of amidacloprid on bee behavior have been already demonstrated. Here we present influence of sublethal doses of amidacloprid on outbreak of digestive disease caused by *Nosema* sp. Four day old bees are initially fed in cages by infective nosema solution at spore counts that do not cause disease in every bee. Bees are then fed chronically with amidacloprid contaminated sugar solution. A group of bees fed with uncontaminated sugar solution served as a control. Bees are sacrificed to examine presence of spores in the mid guts after 12 days. The number of diseased bees and spore counts are recorded. The combined effect of nosemosis and amidacloprid treatment on honey bees is discussed.

Transborder tradings in honey bees

*Per Kryger**

University of Aarhus, DJF, Research Centre Flakkebjerg, 4200 Slagelse, Denmark

*Author of correspondence: per.kryger@agrsci.dk +45 8999 3629

Within the EU common rules for shipping bees between the member states, and for sending bees out of the EU (export) and getting bees into the EU (import). The main piece of legislation is the EU Directive 92/65. See the details here:

http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31992L0065:EN:HTML

Transport inside the EU the transport of animals is free. However, it dependent on a healthy state of the animals, and that they are originating from an area without quarantine restrictions. For American and European Foulbrood the area is a 3 km radius, for small hive beetles (*Aethina tumida*) and Tropilaelaps mites it is a 100 km radius. It is the duty of the member states, and their respective Authority, to enforce these rules. To send bees out of your country, first register as an exporter of bees with your local Department of Agriculture. The shipment of bees, must be announced at least four working days in advance to the Regional Veterinary Authority.

A TRACES certificate must be issued for each shipment of bees, and accompany the bees while in transit. The issuing of a TRACES certificate is a matter for the Regional Veterinary Authority, however, at least in my country you can access the TRACES webside via:

https://sanco.ec.europa.eu/traces/

For you to receive bees from another EU member country, legally, you must register with you Country Authority as an Importer of bees. You will have to notify your authority one working day before arrival that a shipment is due.

New data on bee mortality in France

*Yves Le Conte**

INRA, UMR 406 Abeilles et Environnement, Laboratoire Biologie et Protection de l'abeille, Site Agroparc, Domaine Saint-Paul, 84914 AVIGNON Cedex 9, France.

*Author for correspondance : leconte@avignon.inra.fr

Bee mortality data available from the last year in France will be provided.

Survival of strains of naturally varroa tolerant honeybees in France will be presented. Those bees survive to the mite without any treatment and since at least 7 years on average. Those strains of bees have been included in the program on vitality test of the working group 4. We will give an update of those bee populations and we will discuss how to test them in different environments and different European countries.

Nosema ceranae and Deformed Wing Virus infections of honey bees in a hoarding cage experiment

Marco Lodesani[1], Cecilia Costa[1,], Lara Maistrello[2] and Peter Neumann[3]*

[1]CRA-Unità di ricerca di apicoltura e bachicoltura, Bologna, Italy

[2]Department of Agricultural & Food Sciences, University of Modena and Reggio Emilia, Via G. Amendola 2, 42100 Reggio Emilia, Italy

[3]Swiss Bee Research Centre Agroscope Liebefeld-Posieux Research Station ALP
Schwarzenburgstrasse 16 CH-3003 Bern Switzerland

*Author for correspondance:

The multifactorial nature of colony losses is widely accepted, although synergistic effects of possible causative factors are not completely understood. To investigate interactions between two serious and wide-spread pathogens of honey bee colonies, Deformed Wing Virus and *Nosema ceranae*, an experiment was set up in which worker bees were infected with known amounts of *N. ceranae* spores. To reduce the impact of *Varroa destructor* an apiary was established in an isolated mountain area and mite levels kept as low as possible.

Newly emerged worker bees were kept in 14 hoarding cages (30 bees each). Each cage was provided with 0.2 ml 50% w/v sucrose solution containing 20×10^6 *N. ceranae* spores per ml. Oral infection was therefore obtained via bulk feeding. The number of dead bees in each cage was recorded for ~30 days. After 14 days from beginning of experiment, five live worker bees were removed from each cage. Midguts were removed for spore counts and at the same time, head and thorax of each worker were removed and subsequently analysed for quantitative DWV analyses using standard RT PCR protocols.

The data show a strong positive correlation between the number of *N. ceranae* spores and the number of DWV copies, suggesting that the two pathogens may interact synergistically. *N. ceranae* may enable oral infection by the virus by perforation of the midgut epithelium, or increase replication of the virus by suppression of the immune system.

Israeli acute paralysis virus (IAPV): Turning reciprocal host-virus dynamics into an applicative anti-viral approach

*Eyal Maori, Rita Mozes-Koch, Milena Perman and Ilan Sela**

The Hebrew University of Jerusalem, Faculty of Agriculture, Smith Institute for Plant Sciences and Genetics, Virus Laboratory, Rehovot 76100, Israel

*Author for correspondence: sela@agri.huji.ac.il

Bee colony losses, in particular Colony Collapse Disorder (CCD), have become a major global economic concern due to the dominant pollination contribution of these insects to a wide range of food crops. IAPV is a bee-affecting dicistrovirus which has been strongly associated with CCD. A segment of IAPV was found to be incorporated into the bee's genome and bees harboring an integrated viral segment exhibit a virus resistant phenotype. The exchange of genetic information between IAPV and its host is reciprocal and a bee sequence was found fused to IAPV defective-RNA (dRNA) within purified virus particles. IAPV virions also carry other types of dRNAs: Some of them are recombinants of different genomic parts of IAPV and others are recombinants of IAPV and another dicistrovirus RNA. Interestingly, among some of the dRNAs population the sense oriented strand has recombined with its complement forming hairpin and stem-loop structures. Finally, we report on restraining IAPV infection by feeding bees with double-stranded RNA (dsRNA), as an efficient and applicative way of controlling this viral disease. The possible dynamics of reciprocal sequence exchange between IAPV and its host leading to association with CCD, as well as the potential of controlling viral diseases and CCD by recruiting RNAi-technology are discussed.

Horizontal transmission of Nosema ceranae from worker honeybees to queens

Raquel Martín-Hernández[1], Aranzazu Meana[2], Mariano Higes Pascual*[1],

[1]Regional Apicultural Centre, JCCM, Spain.
[2] Faculty of Veterinary Medicine, UCM, Spain.

*Author for correspondance: mhiges@jccm.es +34 949 25 00 26

Horizontal transmission from worker honey bees to queens is confirmed in a laboratory essay as a possible route of *Nosema ceranae* infection.
An intentionally loss of the queen was provoked in a healthy honey bee colony of *Apis mellifera iberiensis*. These cells were removed and kept in a incubator. At the same time, a frame of sealed brood was obtained from the same uninfected colony and it was kept in identical conditions in a different incubator. The emerging worker bees were removed, confined to cages and kept in a third incubator at 33ºC. Purified *N. ceranae* spores (viiability of 99%) were obtained from infected caged worker honey bees in order to perform infections. Ten replicates of thirty newborn workers (24 hours old) were starved for five hours and caged. Afterwards, five of the replicates were collectively exposed to a total dose of 150,000 spores of *N. ceranae* (approx. 5,000 spores per bee). The other five replicates were fed with the same food with no spores (control replicates). After worker bees consumed the total dose of spores, the bees were transferred to new cages and one queen cell was introduced and they were left until the queen was born and they were examined until the queen was accepted by the nurses. The infected and uninfected replicates were maintained in two different refrigerated incubators at 33ºC. Spore detection and counting was performed individually in every dead bees and at 21days post infection in all the living bees and in 4 queens from each group (n=4 infected and n=4 uninfected) and *N. ceranae* species confirmed by duplex PCR in every macerate. The Histological study was made on one living honey bee from each replicate (n=10) and one living queen from each group (n=2) on day 21 pi. After dissecting out the alimentary canal, tissues were fixed and stained (H&E).
Lesions are only detected in the epithelial ventricular layer of the infected queens and death occurred within three weeks. Our results show that *N. ceranae* affects the ventriculus of the intestinal tract of *A. mellifera* honey bee queens and that this pathogen can be transmitted horizontally from infected worker honey bees to queens, with death occurring within three weeks. The histological study of the infected queens and workers showed similar lesions in the epithelial ventricular layer among them and to those described previously (Higes *et al*., 2007). Indeed, mature spores of *N. ceranae* were only found in the epithelial cells of the ventricle of infected queens and honey bee workers. No spores were found in the epithelium of the crop, proventriculus, Malpighian tubules, small intestine or rectum in workers bees, or in the ovary and fat body of the queens. The presented data may explain the role of house honey bees in natural queen infection.

Role of suboptimal brood rearing temperature in colony losses

Piotr Medrzycki[1,], Gherardo Bogo[1], Simone Tosi[1], Laura Bortolotti[1], Fabio Sgolastra[2].*

[1] Agricultural Research Council, Research Unit for Beekeeping and Silk Breeding, Via di Saliceto 80, 40128 Bologna, Italy
[2] Department of Agroenvironmental Sciences and Technologies – Entomology, University of Bologna, Viale G. Fanin 42, 40127 Bologna, Italy

* Author for correspondence: piotr@inapicoltura.org

The brood rearing temperature is one of the most precisely controlled physiological parameters in a honey bee colony. Due to its importance for colony health, normally only slight deviations from the optimal level may occur. Nevertheless, in particular situations the brood may be subject to conditions of suboptimal temperature, e.g. when the insufficient bee number cannot assure adequate thermoregulation. Moreover, in previous studies, adult bees reared at suboptimal temperature during pupal development, showed decrease in short-term learning and memory capacities. These bees could have difficulties to perform thermoregulation behaviour causing, again, reduced new brood temperature.
To investigate the effects of the brood rearing temperature, a laboratory study was carried out. The larvae were reared *in vitro* at two different temperatures: 35°C (optimal) and 33°C (suboptimal) from the first hours of life until 15 days of age. Larval mortality, adult emergence and adult longevity were measured. Furthermore the susceptibility to the intoxication by Dymethoate was studied both on the larvae and on the adults emerged from the brood reared at the tested temperatures.
The reduction of brood rearing temperature by 2°C had no effects either on larval mortality, or on adult emergence rate, while adult longevity was strongly affected. Moreover adult bees, emerged from brood reared at the suboptimal temperature, responded quicker to intoxication by Dymethoate. Surprisingly, at the lower temperature, the larval LD50 (48h) was much higher and the response to the intoxication was delayed. In fact, after 24 hours, the mortality rate was quite stable at 35°C, while it was still rising at 33°C. This may be explained by the slower larval metabolism at the lower temperature, with the consequent slower active ingredient absorption.
With this study we can conclude that adult honey bees deriving from brood reared at suboptimal temperature have lower fitness and are more susceptible to pesticide intoxication. We hypothesise that a slight poisoning in early spring i.e. in the conditions of low external temperatures, having apparently no important negative effects on the colony, can lead to suboptimal brood temperature, due to insufficient number of bees, and could affect the fitness of the adults that will emerge. Consequently, the next brood, reared by these adults, may be neglected.
Therefore, the low-temperature-brood-rearing should be considered a significant stress factor and may play important role in bee/colony losses, even if its effects on the entire colony are delayed in time and difficult to link directly to the primary cause (slight bee loss in spring).

Productive Traits of Honeybee Lines

Mića Mladenović[1], Nebojša Nedić[2], Ljubiša Stanisavljević[1]*

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia

[2]University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia

*Author for correspondance: mica.mladenovic@gmail.com

The subspecies Apis mellifera carnica Pollman has been examined and confirmed great productive possibility in the territory of Serbia. Apart from reproduction of selection material, it also improves the important morphological and productive features, while paying special attention to productivity, wintering, spring development and resistance to main bee and brood diseases.

The study of morphometric factors of Apis mellifera carnica showed great variability of the domestic subspecies, which has several varieties with good basis for further selection and verification. Different material from different varieties in starting results made it possible to achieve greater success in selecting autochthonous material in the examined areas.

Apart from examining morphometric and productive characteristics of honeybees with tolerance to diseases we have started microscope measuring of queen quality using histological examination of ovaries, artificial insemination, and we are going to attempt to form controlled mating grounds for queens.

We can conclude that form both economical and biological point of view that work on selection is justifiable and successful only if there is variability which is transferred to offspring.

APENET: Network for monitoring honeybee mortality and colony losses in Italy

Franco Mutinelli[1], Anna Gloria Sabatini[2], Albino Gallina[1], Piotr Medrzycki[2], Fabio Sgolastra[3], Laura Bortolotti[2], Claudio Porrini[3]*

[1]Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory for Beekeeping, Viale dell'Universita' 10, 35020 Legnaro (PD), Italy

[2]CRA-API, Bologna, Italy

[3]DISTA, Bologna, Italy

*Author for correspondance: fmutinelli@izsvenezie.it +39 049 8084287

Honeybee and colony mortality have been reported for several years in many countries as well as in Italy. In the last years this phenomenon has become increasingly serious, and several hypotheses have been proposed to explain honeybee and colony losses. Honeybee colony losses are being surveyed in several European countries, but these surveys are not sufficiently structured. Based on beekeepers' reports, honeybee losses in Italy follow a clear seasonal pattern: a) spring and summer colonies loose many foragers due to agrochemicals (bee-losses); b) late summer to winter, the impact of pests (including Varroa) and pathogens becomes more important (colony losses). To assess the extent and investigate the possible causes of honeybee and colony losses in Italy it is needed to establish a national monitoring network. Regional modules composed of five apiaries with ten non-migratory colonies each to ideally cover the national territory will be identified. Colonies will be inspected four times a year (right after winter, spring, summer and just before winter) for several parameters, e.g. health and nutritional condition, colony strength, management practice and appropriately sampled. Data collected will be input in a real-time database available in the web. In addition, dead and live bees as well as several beehive matrices (brood, pollen, wax, honey) will be collected during each inspection for laboratory investigations. The information collected through APENET will provide a broad database for the assessment of the overall health condition of honeybees colonies at national level.

Bee monitoring questionnaire level 3

Bach Kim Nguyen, Jacques Mignon, Eric Haubruge*

Functional and Evolutionary Entomology – Gembloux Agricultural University

*Author for correspondance: nguyen.b@fsagx.ac.be +32(0)81622280

The focus of the working group 1 is to develop a standardized monitoring which could be used by the 35 countries participating in the COLOSS project.

This monitoring could be divided in four levels: the first level is the quantification of the mortality, the second and the third levels are respectively the comparison of the situation in the different regions and countries and the identification of the causative factors. The last level is the development of a system for predicting potential honeybee colony losses.

Then the aim of the bee monitoring level 3 is to try to identify the honeybee mortality cause. A bibliographic study has been conducted and a questionnaire which includes specific symptoms or symptoms related to few potential causes has been written.

The preliminary questionnaire will be presented with complementary analyses which should be conducted. Moreover the difficulties related to this questionnaire will be cited and explained.

The Organisation of Turkish Beekeepers for Monitoring System

*Asl› Özk›r m**

Hacettepe University Department of Biology Bee Health Laboratory 06800 Beytepe-Ankara/TURKEY

* Author for correspondance: ozkirim@hacettepe.edu.tr +90 312 2978043

Turkey has an important role in Beekeeping Industry. It has approximately 4 millions colonies and 150.000 family live with only beekeeping inputs. There are 3 million migratory beekeeping colony(75% of total honey bee colonies).Turkish Beekeepers go to 3 different geographic location every year and transport their colonies 2000 km. Major Honey Bee Diseases: AFB, EFB, Varroasis, Nosemosis. Pesticides (Imidaclopride??),Starvation,Incorrect Application for treatment of Honey bee diseases,New pests and pathogens (viral infections and N. ceranae),Old queen,The quality of comb foundation and Climatic changes

Colony losses in 2007:8-10%, in 2008: 1.8%, in 2009: 0.7% (up to now) respectively.

They also produce 85% of pine honey production of the world. Turkey has 81 cities and in 76 of them Turkish Beekeepers Association was founded in 2003. All City Beekeepers Associations are managed by Turkish Central Beekeeper's Association. Since 2003, Turkish Agriculture Ministry has given some responsibilities to the Central Turkish Beekeepers Association .Every beekeeper who has at least 50 colonies has to register to their city's Beekeepers Assoc.Being a member of a Beekeepers Assoc. Give the beekeepers some advantages. By this way, we obtain huge level of motivation to be answered questionnaries or pick up samples from beekeepers. Each member has an ID number for beekeeping and lots of plaque which is equal with their colony number. Turkish Central Beekeepers Assoc.+Turkish Agriculture Ministry+ Hacettepe University have worked together since 2003. %92 of beekeepers are registered for now. All beekeepers have plagues contains barcode number for each colonies. Begin to use barcode system gives a big oppurtinity to follow colonies and honeys from hive to honey jar. Our new aim is entegration of the Honey Bee Health Monitoring System to this registration system.

Vitality and productivity of local Bulgarian honey bees

Plamen Pavlov Petrov[1], Evgeniya Neshova Ivanova[2]*

[1]Agricultural University – Plovdiv

[2]University of Plovdiv "Paisii Hilendarski"

*Author for correspondance:geneiv@uni-plovdiv.bg

A new actualized and detailed program for bee selection and queen rearing in Bulgaria was developed and approved by the Ministry of Agriculture in 1999. The main goal of that program is to prevent the gene fund (search, reproduction and distribution) of the local Bulgarian honeybee. The local honey bee type (*A. m. rodopica*, Petrov, 1990) is productive enough and well adapted to the local conditions. Colony losses during last years are between 5 – 10%. The breeding and reproduction in apiculture is connected with the selection and choice of mother and father colonies which biological and productive qualities are controlled. A precondition for breeding process is the identification of the origin of the bee colonies by morpho-ethological analysis and the available stock documents. The biological and productive quality control includes determination and estimation of the queen fertility – by the brood quantity and quality, winter resistance, swarming inclination, aggressiveness level, honey and wax productivity, hygienic behaviour. For thoroughbred breeding, bee colonies are selected with high queen fertility, high honey productivity, good winter resistance, low aggressiveness, good hygienic behaviour. For the needs of the beekeepers, the reproduction of selected queen bees is made in registered reproduction apiaries for queen and swarm rearing. For characterization of queen quality, the following parameters are measured in the scientific center's laboratory of the National Apiculture Breeding Association: live weight (mg) of unfertilized queens; live weight (mg) of fertilized queens; density (capacity) of worker brood; average fecundity for twenty-four-hour period; the diameter of spermatheca. The live weight should not be less than 190 mg for unfertilized queens and 230 mg for fertilized queens. The diameter of the spermatheca should not be less than 1.2 mm. The queen should lay more than 2000 eggs for 24 hours during the period before the main pasture. The frame used for studying of worker brood density should contain not more than 10% empty cells.

Statistical data of Agricultural Ministry and National Bee Breeding Association show that honey productivity per a colony is about 20 kg on the average. Honey productivity in North-east and South-east of Bulgaria is 40-50 kg/colony and in mountain regions of the country – about 10 – 20 kg/colony.

Oxalic acid: Toxicology on *Apis mellifera*

Eva Rademacher , Marika Harz*

Freie Universität Berlin Institut für Biology

Königin-Luise-Str. 28-30 D- 14195 Berlin

*Author for correspondance: radem@zedat.fu-berlin.de

Varroa destructor plays an important role in the phenomenon of bee colony losses. Combating this parasite is essential: Oxalic acid in its approved formulation as veterinary drug for honey bees is used as routine treatment but the mode of action is not yet clear. The acute lethal toxicity and sublethal effects on bees were tested after oral application resp. dermal application in the laboratory by individual treatment of the bees. Dermal application of oxalic acid dihydrate solution showed very low bee mortality in the range of the controls. Oral application resulted in a much higher bee mortality. As the mortality in the field is very low, oxalic acid seems to act as a contact poisoning without considerable oral intake by the bees.

With regards to sublethal effects, the detection of oxalic acid in the haemolymph will give an indication of pharmacodynamics of the substance. The analysis is still in process.

Chronic bee paralysis virus: dissemination in honey bee colonies and diagnosis

Ribière, M*.; Blanchard, P.; Schurr, F.; Celle, O.; Chauzat, M.P.; Faucon, J. P.

Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Les Templiers, Route des Chappes, BP 111, 06902 Sophia Antipolis, France.

*Author for correspondance :m.ribiere@afssa.fr

The Chronic bee paralysis virus (CBPV) is the aetiological agent of an infectious and contagious disease of adult honey bees known as chronic paralysis. Over the past few years, the outbreak in France of trembling symptoms caused by CBPV has led our laboratory to conduct studies in order to improve the knowledge on this agent and on the disease. Full-length nucleotide sequences for the two major RNAs of CBPV have been characterized, leading to the development of, firstly, molecular diagnostic tools that can be used to detect genetically variable viral isolates and, secondly, a Real-Time PCR viral quantification technique. This two step real-time RT-PCR assay allowed us to quantify CBPV genome and to determine the distribution of CBPV infection within hives and their environment. Samples of various ages of brood and adult bees were collected from several hives at different time of the year (spring, summer, and autumn). The virus was detected in all the live stages and along the year. Moreover, significant high mortality rates were observed in France during the 2007 and 2008 beekeeping season. Bee samples from apiaries located in various parts of France were analysed to evaluate the CBPV load by Real-Time PCR. Some surveyed apiaries presented high viral loads confirming the diagnosis of the chronic paralysis and highlighting the role of CBPV in bee mortalities. Knowledge on CBPV genome, sequence and variability, has allowed us to develop tools to better follow virus dissemination, including bee faeces and *Varroa destructor,* and ways of spread. We have detected CBPV, for the first time, in two species of ants (*Camponotus vagus and Formica rufa*). These results suggest ways by which the infection may be spread and other sites of viral persistence in the apiary environment.

Monitoring colony losses in Bosnia and Herzegovina

Violeta Santrac, Goran Mirjanic*

Bosnia and Herzegovina

Active colony losses monitoring was undertaken on the whole territory in Bosnia and Herzegovina. Thanks to the COLOSS project opportunities, created and slightly optimized questionnaire protocol were distributed to the all local beekeeping associations. Also, national beekeeping magazine was part of information promotion in planed monitoring and way how we want competently disseminate questions and share interests between science and beekeepers because currently no extension services in BIH beekeeping practice. Also results about losses were compared with national veterinary body which had reporting system based on obligatory and voluntary notification response to honey bee disease.

Telephone interviews were used as second level of information sources from beekeepers that reported loses more higher than technological average limit is acceptable. During the survey we checked pathogen situation based on varroa protocol control, *Nosema cerane* presence and virus presence in honey bee population.

Some significant info we enhance from survey under varoa control chemical protocol that is not officially proposed and is indicator of creativity in beekeeping practice because in Bosnia there are no strict anti varroa drug regulations on the market.

Final results will be presented more clearly in poster presentation.

Wing vein anomalies in breeding colonies

Enikö Szalai-Mátray[1], L. Békési[1], T. Szalai[2]*

[1]Research Institute of Animal Breeding and Nutrition Research Group for Honeybee BreedingH-2100 Gödöllő, Meheszet
[2]Szent István University, Inst. of Environmental and Landscape Management Gödöllő

*Author for correspondance: matray@katki.hu   +36 30 210 8899

Wing vein anomalies are frequently in worker bees and drones especially in the cubital cell. Missing veins or extra ones often occur. Cubital index is an important characteristic of the races. This index is a base for race identification in breeding. Vein breaking, elongation or new vein formation can also occur. The inheritability of the latter has not been proven yet. Delta-shape and vein breaking (especially at the cubital cells) is often of hereditary origin.

While measuring the cubital index of the right front wing in one bee colony (48 simples), relatively many abnormal cells and veins can be observed around the area cubital, area discoilis and the transversocubitalis veins.

We found new abnormalities, new vein on the transversocubital vein; extenuation or brake on the transversocubital vein, and new cell defects appear, new cell initiative on the border of the cubital area; new vein initiative on the transversocubital vein; brake on t_2 transversocubital vein; missing on t_3 transversocubital vein, as well as breaking and diminishing on the d_2 transversocubital vein were detected.

In 2001-2007 wing vein anomalies varied between 4-27% in the tested breeding colonies of 38 stations (22 stations – 6 years, 7 in 5 years and 4 in 4 years.)

Owing to the concentration of bee colonies (9-10 colony/km²), isolation (in a circle with 20-30 km radius) - is necessary for the breeding - can be realized only in wildlife protection areas in Hungary.

Role of food quality in bee response to pesticides

Simone Tosi[1,], Piotr Medrzycki[1], Gherardo Bogo[1], Francesca Grillenzoni[1], Laura Bortolotti[1], Fabio Sgolastra[2].*

[1] Agricultural Research Council, Research Unit for Beekeeping and Silk Breeding, Via di Saliceto 80, 40128 Bologna, Italy
[2] Department of Agroenvironmental Sciences and Technologies – Entomology, University of Bologna, Viale G. Fanin 42, 40127 Bologna, Italy
* Author for correspondence: st29@libero.it

Pollen is the honey bee's main protein supply. Newly emerged bees need pollen alimentation to guarantee correct development of physiological conditions and breeding potential. To ensure functional and efficient adult bees not only the quantity but also the quality of pollen is important. Pollen of different plant species vary in nutritional quality for honey bees. Previous studies evidenced that the pollen mixture, instead of monocultural pollen, is the most adequate food for bees. The kind of pollen collected by bees is in close relationship with the vegetational spectrum of the hive surrounding area. Thus, if the bee colony is surrounded by areas characterised by intensive agriculture (e.g. Po Valley in Italy), it may mainly collect monocultural pollen. Moreover, in these areas, pesticide treatments are normally frequent.
The present study was based on the hypothesis that the quality of pollen available for the honey bee colony may influence the bee susceptibility to the intoxication by pesticides. For this reason, the same pesticide treatment could cause negligible or significant damages (even the colony loss) basing on the availability of high quality pollen.
In the experimental apiary, in order to obtain newly emerged bees of the same age, the queen bee was isolated on a comb for about 30 hours in a queen-excluding cage. Subsequently the queen bee was removed from the cage and the comb was left isolated for other three days, to avoid further egg laying. The comb was incubated inside the beehive for 20 days in order to guarantee the most natural conditions, then it was moved to an emerging cage and kept at 34,5 °C. The bees were incubated at this temperature until the end of the test. Two days after the emergence beginning, the bees were divided into three groups and fed *ad libitum* with water, organic *Robinia* honey and one of three kinds of pollen according to the group: *Phacelia* (high quality), *Prunus* (lower quality) or a mixture of pollen (20% *Phacelia*, 20% *Prunus*, 20% *Papaver*, 20% *Cruciferae* and 20% *Amorpha*). Thus, three groups of bees provided with three different pollen diets were obtained. On the 9th day, the bees were divided into groups of 20 individuals in small test cages where Clothianidin (a neonicotinoid pesticide considered one of the possible causes of the recent colony losses) in sucrose syrup (50%), was administered at the following doses: 0, 1, 2, 4, 8 and 16 ng per bee. Once the bees consumed completely the test solution, sucrose syrup (50%) was supplied *ad libitum.* Bee mortality after 3, 6, 12, 24, 48 and 72 hours from the Clothianidin administration was recorded and the LD50 of the a.i. was calculated in relationship to the alimentation quality.
In this way the influence of the quality of protein nourishment provided during the first days of adult life on the response to intoxication by Clothianidin was evidenced.

Genetic diversity of honey bees (*Apis mellifera* L.) in Turkey revealed by RAPD markers

Tunca, R.I.[1,2], Koleoglu, G.[1], Atagan, Y.[1], Kence, M.[1]*

[1] Middle East Technical University, Department of Biology06531 Ankara, Turkiye

[2] Yuzuncu Yil University, Agriculture Faculty, Dept. of Animal Science Van, Turkiye

* Author for correspondence:rivgin@gmail.com +90 505 2544852

The honey bee (*Apis mellifera* L.) is ecologically and economically important insect species. Five of 26 identified subspecies are distributed in Turkey. It is essential to determine and preserve the genetic variation which is especially a valuable resource at this time of global honey bee losses. RAPD markers were used to assess the genetic diversity in 360 colonies from 25 provinces. In a total of 720 worker bees, ten RAPD primers amplified 105 bands, all of which were polymorphic. Mean gene diversity values (He) ranged between 0.035 and 0.175, G_{ST} values 0.060-0.395, and the private band patterns reflected a high level of genetic variation. AMOVA analysis partitioned the genetic variation as 60% within populations, 40% among populations. The Mantel test did not reveal significant correlation between the genetic and geographic distances. Neighbor-joining analysis showed that the bees of Thrace region of Turkey and an island relatively close by clustered together. The results showed that the RAPD markers are good to discriminate the honey bee populations and provide appropriate information for conservation plans.

Diversity of Honey Bees (*Apis mellifera* L.) On The Territory of Republic of Macedonia

Aleksandar Uzunov, Hrisula Kiprijanovska and Sreten Andonov*

[1] Faculty of Agricultural Sciences and Food - Skopje, Republic of Macedonia

*Author for correspondance:

Morphometric analysis was undertaken and performed on bee samples taken from 6 different bee yards (Kavadarci, Skopje, Bitola, Gevgelija, Vinica and Mavrovo) located at the territory of the Republic of Macedonia. The purpose of this research was to determine the diversity of the honey bees, as well as to verify the relationship of these bees with the other honey bee subspecies. The morphometric research was performed on the wing venations at the right front wing of the bees (n=1800) and through this research data was obtained for the values for a total of 21 characteristics. The bees were examined according to the bee yards from which they originate (n=6) and in equal groups (n=300). The analysis of the values of the characteristics of the analyzed bees is deducted through descriptive statistics, analysis of the variants (ANOVA) and testing of the relation of the analyzed honey bees with the standards of the most important honey bee subspecies.

It was determined that the bees from the analyzed bee yards were characterized with distinctive mutual variability and diversity, and it was also concluded that this variability decreased with the increase of the altitude and vice versa. The subspecies relationship of the total sample of analyzed bees (n=1800) expressed in percentage is: Apis mellifera macedonica - 15.87%, Apis mellifera ligustica - 12.70%, Apis mellifera carnica -10.32%, Apis mellifera caucasica - 4.76% and Apis mellifera mellifera - 4.76%. This variability and diversity is solid basis for selection of large number of genotypes with desired biological and production characteristics for introducing National beekeeping breeding program in Republic of Macedonia.

Beehives monitoring on lavender nectar flow in France 2008

J. Vallon, P. Jourdan, L. Belzunces, J. Gardette, A. Kretzschmar

[1]ADAPI Association pour le développement de l'apiculture provençale, 22 avenue Henri Pontier, 13 626 Aix en Provence cedex 1.
[2]INRA Abeilles et Environnement, Biostatistiques et Processus spaciaux, Agroparc, 84 914 Avignon

*Author for correspondance : j.vallon.adapi@free.fr

Lavender nectar flow in France is known to fall down bee hives population in France because the queen stop laying and daily mortality increase compared with behaviour on mountain apiary.
Since 5 years, professional beekeepers observed early fall down: colonies with decreased populations without mortality around the hives. The phenomenon does not occur on the same location and honey production varies into apiary but also between close apiaries.

Beehives monitoring on 16 apiaries all around lavender areas in south east France was developed by the Abeilles et Environnement lab in INRA. Weight gain, estimated bees population and sealed brood from frame photos, lavender flowering were measured and lavender flowers or bee's samples were collected to explain beehives evolution.

In order to limit toxicology and pathology analysis number we have to describe apiaries or hives with abnormal behaviour. The Biostatistiques et Processus spaciaux lab in INRA use the adapted Bühlmann index to integrate bees population and sealed brood on 2 different period of the nectar flow and compare it with a theoretical index without mortality. The analysis of index distribution allows us to identify hives with normal or extreme behaviour that leads us to choose sample for analyse.

Implementation of the Coloss Questionnaire version 1

*Romée van der Zee**

NCB Netherlands Centre for Bee Research Durk Dijkstrastr. 10, 9014 cc Netherlands

*Author for correspondance: romee.van.der.zee@beemonitoring.org

One of the aims of Coloss is to collect data on colony losses between countries and over the years. As a result of discussions in Coloss workgroup 1, the first edition of the basic questionnaire was proposed at the Coloss conference in Zagreb. In April 2009, after some fine tuning, the tool was ready for implementation. Austria, Turkey, The Netherlands, Belgium, Spain, Italy, France (professional beekeepers), United Kingdom, Norway, Denmark, Sweden, Poland, Portugal, Hungary, Rep. of Macedonia, Germany, Israel, Croatia and Ireland have decided to implement the questionnaire.
In April 2009 some countries had already a questionnaire running, others decided to start the coloss questionnaire spring 2010. Countries that used the Coloss questionnaire like the Netherlands Denmark/Sweden , were positive about the outcome. At the workshop in Amsterdam improvements were discussed, especially a better discrimination between (1) late summer and winter losses, and (2) adding a question about queen problems.
This has resulted into a second version of the Coloss questionnaire, to be finalized during the Coloss conference in Montpellier.
Funding for the implementation is poor in most countries. In many countries beekeeper associations have to provide fundings and sometimes take a rather indecisive or negative attitude towards it. The motivation by beekeepers to fill in questionnaires in general is rather low, with the effect that a lot of effort is needed to come to a representative result. This is a vulnerability for the monitoring results that has to be met in the future.

Effect of temperature on virulence of fungal pathogens in honeybees

Svjetlana Vojvodic[1], Annette Bruun Jensen[1], Jacobus .J. Boomsma[2], Jørgen Eilenberg[1]*

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

[2] Centre for Social Evolution, Department of Biology, Universitetsparken 15, 2100 Copenhagen, Denmark

*Author for correspondance: svo@life.ku.dk + 45 35 33 26 78

Environmental temperature is known to have a crucial role in mediating the outcome of host- parasite interactions. I have investigated the impact of temperature on host-pathogen dynamics, using honey bee larvae as host, and the causative agent of chalkbrood disease – the fungus *Ascosphaera apis,* as pathogen.

The normal temperature of a bee hive is 34 °C. In this study it was observed that in vitro reared honey bee larvae were more susceptible to chalkbrood infection after a 24 hour temperature drop to 27 °C, than if kept constant at 34 °C.

Contrary to chalkbrood, the opportunistic fungus *Aspergillus flavus* was observed to kill significantly less (*P* < 0.001) when larvae were cooled to 27 °C for 24 hour.

These results suggest that chalkbrood and *A. flavus* operate with different temperature strategies.

Situation of Nosemosis in Turkey

Aygün Yalç nkaya, Nevin Keskin*

Hacettepe University Department of Biology Bee Health Lab. Beytepe

Ankara/TURKEY

*Author for correspondance:aygun@hacettepe.edu.tr +90 312 3978011

Turkey's geographical position and properties give rise to three different climate regions. Climatic conditions and geographic properties are very effective at distribution of diseases. Honey bee diseases, especially Nosemosis, disperse in a wide range all over the Turkey.

In our research, adult honey bees collected from Mediterranean Region (South Anatolia), Black Sea Region (North Anatolia), Aegean Region (West Anatolia), and East Anatolia Region in spring and autumn. Samples were brought to the lab. in a short time. All intestines were removed and homogenized. 1 ml de-ionized water per bee was added to the homogenate. *Nosema* spp. spores in 0,1 ml final homogenate have been counted by using Neubauer-counting chamber under light microscope.

Nosemosis levels have been graded according to *Nosema* spp. spore numbers.

Consequently, Nosemosis has been seen at high levels in Black Sea Region which has humid and warm conditions. Contrary to Black Sea Region Nosemosis level is below the normal levels in Mediterranean Region. Mediterranean Region has very hot air conditions. Finally, Nosemosis is at normal levels in East and West Regions. East and West Regions have average climatic conditions.

Furthermore, during the examinations, *Nosema* spores were determined in different sizes. Also, *Nosema ceranae* research has begun in our honey bee health laboratory.

LIST OF CONTRIBUTIONS

P = Poster

CT = Contributed talk (15 min at plenary session)

S = Short Talk (5 min at WG meetings)

		Member		**Contribution**	**Title**
	1	Allier	Fabrice	P	Colonies losses during winter 2008/2009
	2	Andonov	Sreten	P	Diversity of Honey Bees (Apis mellifera L.) on the Territory of Republic of Macedonia
	3	Bienkowska	Malgorzata	P	European test on genotype - environment interactions
	4	Blacquière	Tjeerd	P	Timing varroa control affects winter bee survival
	5	Bogo	Gherardo	P	Role of temperature related to bee age in the response to pesticides
	6	Bouga	Maria	P	Colony losses and Genetic Diversity of Honey Bees in Greece
	7	Brodschneider	Robert	P	Colony losses in Austria, a 2 years survey
	8	Bubalo	Dragan	P	The influence of the drones to the varroa tolerability
WG4	**9**	**Büchler**	**Ralph**	**S**	**European test on genotype - environment interactions**
	10	Charrière	Jean-Daniel	P	Colony losses in Switzerland: newest results
	11	Chauzat	Marie-Pierre	P	A case control study and a survey on mortalities of honey bee colonies (Apis mellifera) in France ……..
	12	Chejanovsky	Nor	P	Progressing Survey of Honeybee Colony Losses in Israel
	13	Coffey	Mary	P	Monitoring colony losses in Ireland
	14	Costa	Cecilia	P	Vitality parameters in commercial honey bee colonies
WG1	**15**	**Crailsheim**	**Karl**	**S**	**Colony losses in Austria, a 2 years survey**
	16	Dahle	Bjorn	P	Colony losses in Norway 2008- 2009
	17	Dainat	Benjamin	P	Ants and mites: where is the link?
	18	Dietemann	Vincent	P	Small cells against Varroa: filling the gaps

	19	Drazic	Maja	P	The influence of the drones to the varroa tolerability
CT	**20**	**Ellis**	**Jamie**	**CT**	**CCD Research in the United States**
	21	Filipi	Janja	P	The influence of the drones to the varroa tolerability
	22	Gajda	Anna	P	Most probable causes of colony losses during the winter of 2008/2009 in Poland
WG2	**23**	**Genersch**	**Elke**	**S**	**Evaluation of the virulence of deformed wing virus (DWV) for honey bees.**
	24	Hartmann	Ulrike	P	The neglected majority: common bacteria can induce mortality in adult honeybee workers
	25	Harz	Marika	P	Toxicology of oxalic acid on Apis mellifera
	26	Hatjina	Fani	P	Comparison between anatomical/ physical characteristics of queens and the performance of their sisters ....
	27	Hegic	Gordana	P	The influence of the drones to the varroa tolerability
	28	Higes	Mariano	P	Horizontal transmission of Nosema ceranae from worker honeybees to queens
	29	Ivanova	Neshova Evgeniya	P	Bulgarian honeybee colony losses during 2008/2009; Vitality and productivity of local Bulgarian honey bees
	30	Ivgin Tunca	Rahsan	P	Genetic diversity of honey bees (Apis mellifera L.) in Turkey revealed by RAPD markers
WG2	**31**	**Jensen**	**Annette Bruun**	**S**	**Susceptibility of different honey bee subspecies: Apis mellifera carnica, A. m. ligustica and ....**
	32	Kence	Aykut	P	Honey bee colony losses in Turkey and their possible causes
	33	Kence	Meral	P	Genetic variation in honeybee populations:a valuable resource at the time of global bee losses
	34	Kezic	Nikola	P	The influence of the drones to the varroa tolerability
	35	Kiprijanovska	Hrisula	P	Diversity of honey bees (Apis mellifera L.) on the territory of Republic of Macedonia
	36	Korpela	Seppo	P	Nosema situation in Finland
WG3	**37**	**Kralj**	**Jasna**	**S**	**Influence of sublethal doses of imidacloprid on break out of nosemosis**
	38	Kristiansen	Preben	P	Colony losses in Sweden – latest data
WG4	**39**	**Kryger**	**Per**	**S**	**Transborder tradings in honey bees**
WG1	**40**	**Le Conte**	**Yves**	**S**	**New data on bee mortality in France**
WG2	**41**	**Lodesani**	**Marco**	**S**	**Nosema ceranae and Deformed Wing Virus infections of honey bees in a hoarding cage experiment**
	42	Martín-Hernández	Raquel	P	Horizontal transmission of Nosema ceranae from worker honeybees to queens
	43	Meana Manes	Aranzazu	P	Horizontal transmission of Nosema ceranae from worker honeybees to queens
WG3	**44**	**Medrzycki**	**Piotr**	**S**	**Role of suboptimal brood rearing temperature in colony losses**
CT	**45**	**Meikle**	**William G.**	**CT**	**The enemey of my enemy: treating bees with entomopathogenic fungi against varroa mites**

	46	Meixner	Marina		European test on genotype - environment interactions
	47	Mihaljevic	Ivan	P	The influence of the drones to the varroa tolerability
	48	Mirjanic	Goran	P	Monitoring on honey bee loses and some pathogens in BIH 2008/2009
	49	Mladenovic	Mica	P	Productive traits of honeybee lines
	50	Moosbeckhofer	Rudolf	P	Colony losses in Austria, a 2 years survey
CT	**51**	**Moritz**	**RFA**	**CT**	**Is breeding for disease resistance a sustainable concept in honeybees?**
	52	Murilhas	Antonio		European test on genotype - environment interactions
	53	Mutinelli	Franco	P	APENET: Network for monitoring honeybee mortality and colony losses in Italy
plenary	**54**	**Navajas**	**Maria**	**S**	**Local organisational matters**
CT	**55**	**Neumann**	**Peter**	**CT**	**Introduction and organisational matters**
	56	Nguyen Bach	Kim	P	Bee monitoring questionnaire level 3
	57	Ozkirim	Asli	P	The Organisation of Turkish Beekeepers for Monitoring System
	58	Panasiuk	Beata	P	European test on genotype - environment interactions
	59	Petrov	Plamen	P	Vitality and productivity of local Bulgarian honey bees; Bulgarian honeybee colony losses during 2008/2009
CT	**60**	**Pettis**	**Jeff**	**CT**	**Colony losses in the US**
	61	Rademacher	Eva	P	Toxicology of oxalic acid on Apis mellifera
	62	Ribière Chabert	Magali	P	Chronic bee paralysis virus: dissemination in honey bee colonies and diagnosis
WG1	**63**	**Ritter**	**Wolfgang**	**S**	**WG1 Organisation**
	64	Santrac	Violeta	P	Monitoring on honey bee loses and some pathogens in BIH 2008/2009
	65	Sela	Ilan	P	Israeli acute paralysis virus (IAPV): Turning reciprocal host-virus dynamics into an applicative anti-viral ....
	66	Soroker	Victoria	P	Progressing Survey of Honeybee Colony Losses in Israel
	67	Stanisavljevic	Ljubisa	P	Productive traits of honeybee lines
	68	Szalai	Tamas	P	Wing vein anomalies in breeding colonies
	69	Szalai-Matray	Enikö	P	Wing vein anomalies in breeding colonies
	70	Svecnjak	Lidija	P	The influence of the drones to the varroa tolerability
	71	Tomljanovic	Zlatko	P	Possible reasons for colony losses in Croatia
	72	Topolska	Grazyna	P	Monitoring of winter (2008/2009) honey bee colony losses in Poland

WG3	**73**	**Tosi**	**Simone**	**S**	**Role of food quality in bee response to pesticides**
	74	Uzunov	Aleksandar	P	Diversity of honey bees (Apis mellifera L.) on the territory of Republic of Macedonia
	75	Vaccari	Giacomo	P	Colony losses in the Italian region Emilia-Romagna during summer 2008 and winter 2009
	76	Vallon	Julien	P	Colonies Monitoring on Lavendar: Searching for unusual depopulation
	77	van der Steen	Sjef	P	Impact of a Varroa parasitation on honeybee hemolymph proteins
WG1	**78**	**van der Zee**	**Romée**	**S**	**Adaptation of the basic coloss questionnaire**
	79	Vojvodic	Svjetlana	P	Effect of temperature on virulence of fungal pathogens in honeybees
	80	Wei	Shi	P	Colony losses in China in 2009
	81	Wilde	Jerzy	P	European test on genotype - environment interactions
	82	Yalc nkaya	Aygun	P	Nosemosis situation in Turkey
	83	Yañez	Orlando	P	Effect of vertical transmission on genetic variation of deformed wing virus