



Action FA0803

# Proceedings of the 4<sup>th</sup> COLOSS Conference

Prevention of honeybee COlony LOSSes

in the Faculty of Agriculture  
University of Zagreb  
Svetosimunska cesta 25  
Zagreb, Croatia



SVEUČILIŠTE U  
ZAGREBU



March 3-4, 2009

Dear colleagues

Welcome to the 4<sup>th</sup> COLOSS conference at the University of Zagreb.

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. Kezic and his 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.

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, Thursday, 19 March 2009

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**The Local Organizing Committee for the 4<sup>th</sup> COLOSS Conference**

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Zlatko Puškadija, Damir Šekulja, Ivana Tlak Gajger

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Last update Thursday, 19 March 2009

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## Agenda

Time	Programme
<b>2<sup>nd</sup> March 2009 (Monday) – Hotel Laguna</b>	
16:00 – 18:00	EC Meeting
18:00 - open	Registration, informal social gathering
<b>3<sup>rd</sup> March 2009 (Tuesday) - Faculty of Agriculture University of Zagreb</b>	
07:30	Transportation from the hotel Laguna to Faculty of Agriculture (bus)
08:00 – 09:00	Registration
09:00 – 09:45	Welcome, organisational matters, update on colony losses during the last winter, general information on findings and applications
9:45 – 10:30	Plenary Lecture by JD Ellis “Plight of the Honey Bee: CCD in the U.S.”
10:30 – 11:00	Coffee break with snacks
11:00 – 12:30	Separate WG meetings - reports, detailed planning for 2009
12:30 – 14:00	WG mix: WG leaders together with colleagues, who have this WG as a 2 <sup>nd</sup> choice
14:00 – 15:30	Lunch
15:30 – 16:30	Separate WG meetings - discussion of results from the WG mix
16:30 – 17:00	Coffee break with snacks
17:00 – 18:30	Plenary session – final discussions, planning of the next meeting
19:00 - open	Social event
<b>4<sup>th</sup> March 2009 (Wednesday) - Faculty of Agriculture University of Zagreb</b>	
08:30	Transportation from the hotel Laguna to Faculty of Agriculture (bus)
09:00 – 10:30	Separate WG meetings - tuning of work plans
10:30 - 11:00	Coffee break with snacks
11:00 – 13:00	MC Meeting of the COST Action FA0803
13:00 – 14:30	Lunch
Optional	Afternoon City tour in Zagreb
<b>5<sup>th</sup> - 6<sup>th</sup> March 2009 - Excursion to Unije, depends on weather conditions</b>	
Optional	2 day excursion top the island of Unije (see details in the text below)

Registration on site is required.

**Registration fees: 30 €**

### Excursion - Island Unije

An experimental station for varroa tolerance control and a mating station are situated on the island of Unije since 1998. **Unije** is located in the northern part of the Adriatic Sea and it spreads on only 17 km<sup>2</sup>. The departure from Zagreb to Island Unije is scheduled on 5<sup>th</sup> March 8:00 am. Participants will visit testing apiaries on the island at the same day. Accommodation on the island and meals will be arranged. Participants will return to Zagreb on 6<sup>th</sup> March till the evening.

<b>CONFERENCE LOCATIONS</b>	
<b>Hotel Laguna</b>	<b>Faculty of Agriculture University of Zagreb</b>
Kranjčevićeva 29 10 000 Zagreb Tel: +385 1 3047 000 Fax: +385 1 3047 077 www.hotel-laguna.hr	Svetošimunska 25 10 000 Zagreb Tel: +385 01 2393793 Fax: +385 (0)1 2315 300 www.agr.hr

<b>CONTACT DETAILS OF LOCAL ORGANISERS</b>	
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## Plenary Lecture

### Plight of the Honey Bee: CCD in the U.S.

*Jamie D. Ellis*

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Colony Collapse Disorder (CCD) was first noted in the U.S. in fall 2006. At that time, some beekeepers living in states reporting CCD lost 30-90% of their colonies when 10-20% losses were considered normal. The Apiary Inspectors of America and USDA-ARS estimate that honey bee colony losses for fall/winter 2006-2007 and 2007-2008 were 31% and 36% respectively. These loss estimates are based on phone surveys of beekeepers who manage between 10-18% of the 2.4 million colonies in the U.S. Numerous causes, including CCD, were reported as contributing to the colony losses in both 2007 and 2008. In an attempt to remove the ambiguity surrounding CCD, U.S. bee scientists defined the symptoms associated with the phenomenon. In collapsed colonies, CCD may produce the following symptoms: (1) the complete absence of adult bees in colonies with few or no dead bees in/around colonies, (2) the presence of capped brood, and (3) the presence of food stores that are not robbed by other bees or typical colony pests. CCD symptoms associated with collapsing (weakening) colonies include: (1) an insufficient number of bees to maintain the amount of brood in the colony, (2) the workforce is composed largely of younger adult bees, (3) the queen is present, and (4) the cluster of bees is reluctant to consume food provided to them by the beekeeper. The cause(s) of CCD in U.S. bee colonies remains under investigation. Currently, many conceivable and realistic hypotheses remain plausible. Not listed in any particular order, these hypotheses include, but are not limited to: (1) traditional bee pests and diseases, (2) how the bees were managed, (3) queen source (poor genetic biodiversity), (4) chemical use in bee colonies, (5) chemical toxins in the environment, (6) varroa mites and associated pathogens, (7) bee nutritional fitness, (8) undiscovered/newly-discovered pests and pathogens, and (9) potential synergistic interactions between two or more of the hypotheses. Other hypotheses have been proposed (genetically modified crops, climate change, etc.) but those listed above currently are the most investigated. Considerable funding for CCD research has become available in the U.S. and progress has been achieved on many collaborative research fronts. These include studying the effects of pesticides on bees, understanding the biology of new pests/pathogens, recognizing the effects of environmental and management stresses on bees, etc. In general, there are two large CCD research efforts in the U.S., the first being the USDA Area-Wide project and the second being the BEE CAP project. In addition to these well-funded efforts, many bee scientists in the U.S. have formed partnerships with colleagues nationally and internationally, creating a well-defined, collaborative colony loss research effort.

## Austria

### Colony losses in Austria

*Karl Crailsheim<sup>1,\*</sup>, Rudolf Moosbeckhofer<sup>2</sup> and Robert Brodschneider<sup>1</sup>*

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In Austria no data of colony losses or over winter mortality are published so far. According to data of the Austrian beekeeping association (Österreichischer Imkerbund <http://www.imkerbund.at/index.php/article/articleview/145/1/a5/>), 22198 beekeepers were registered in this organisation in the year 2007, maintaining 278810 colonies. The majority of Austrian beekeepers are hobbyists or sideline beekeepers with low numbers of colonies. The number of big operations is low. In 2008 we visited eight mostly major beekeeping conventions between February 14 and June 28 and handed out a survey and collected it after completion. We conducted a survey among 374 beekeepers in Austria regarding their over-winter colony losses. The questioned beekeepers maintained 16217 colonies in autumn 2007 of which 14059 were still alive in spring 2008. That means a total over winter mortality of 13.3% in Austria. Losses varied from 9.2% in Salzburg to 17.1% in Lower Austria (including Vienna). According to our survey no severe cases of colony losses were reported, although some beekeepers suffered losses higher than the reported total losses. 25.9% of beekeepers lost more than 20% of their colonies. However, total losses were lower than those reported in Poland, particular Regions of Germany, Turkey and the USA. We agree with the beekeepers self evaluation that these losses are mainly due to queen loss, *Varroa* and starvation. Representative data on colony losses for the overwintering period 2008/2009 are generally not yet available at that time of season from Austria. Preliminary results of colony losses during the winter 2008/2009 (5030 colonies checked in February 2009) suggest that until now, losses are not higher, maybe even lower than in 2007/2008. Few reports sent to the Institute for Apiculture in 2008 resembled symptoms described for CCD. But it remains unclear if these symptoms had the same causal agents as CCD in the USA.

## Belgium

### Belgian beekeeping situation and the symptomatology as discriminatory tools

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In Belgium we estimated the numbers of beekeepers to be 8600 but only 2000 beekeepers are currently registered. There is no professional beekeeper in Belgium. The mean number of hives in apiaries is 14. Each two years we are conducting a survey on almost 5% of the registered beekeepers in the south part of Belgium. We visited each apiary, we collected samples of honey, pollen, beebread, wax and honey bees and we studied the potential risk factors by molecular, microscopic, microbiological and chemical analysis. In the apicultural season 2004 – 2005, the mortality rate in apiaries ranged from 0 to 84.2%. The global mortality rate was 16.37%. The mortality occurred between October and April in 92.86% of the cases. In the apicultural season 2006 – 2007, the mortality rate was lower with a percentage of 7.3%. Most of the collapses were still observed in the winter. Great many symptoms were recorded in apiaries and they were not the same in every apiary. These observations confirmed the multifactorial aspect of the honey bee mortality. Symptoms did not allow identifying precisely a cause of the mortality because many symptoms are not specific. Moreover a lot of diseases are asymptomatic. Therefore we analyzed samples in the laboratory and we identified different potential causes. We have identified *Varroa destructor* and associated viruses, the lack of food in the winter and the American foulbrood at the clinical stage. During the season 2007 – 2008, few beekeepers have suffered massive mortality in Belgian apiaries. It is likely that the communication campaign in Belgium about the importance of *Varroa destructor*, the presence of virus, the lack of food in the overwintering colonies allowed limiting their negative effect on the honey bee mortality rate. Currently, laboratories in the north part and south part of the country are collaborating to work out a national strategy to control *Varroa destructor*.



## Bosnia and Herzegovina

### Colony losses in Bosnia and Herzegovina

*Violeta Santrac*

Carniolan bees (*Apis mellifera carnica*, Pollm.), well know world-wide, are autochthonous bees of this area. There are not national beekeeping unit. In this days they negotiate about future union and they exist as two associations: 1. Association of beekeepers of Republic of Serpska; 2. Assotiation of federation union beekeepers of Federation BIH

**Tab.1.** There are *situation data* of two constitutive entities in beekeeping area BIH

R.br.	REPUBLIC OF SEPSKA	No associations	No beekeepers	No hives	Average No hives/beekeeper
1. 1	Hercegovacka	6	586	27976	47.7
2. 2	Potkozarska	8	358	18580	51.9
3. 3	Dobojska	6	388	17834	46.0
4. 4	Sembersko-Majeviska	1	363	10924	30.0
5. 5	Krajiska (BL)	7	284	10067	35.4
6. 6	Bircanska	4	160	5498	34.4
7. 7	Romanijska	4	178	5411	30.4
8. 8	Gornje-drinska	3	162	3885	24.0
<b>TOTAL 2008</b>		<b>39</b>	<b>2 479</b>	<b>100 175</b>	<b>40.4</b>
R.br.	FEDERATION OF BIH	No associations	No beekeepers	No hives	Average No hives/beekeeper
	?	?	?		?
<b>TOTAL 2007</b>		<b>?</b>		<b>175 580</b>	
<b>BOSNIA AND HERZEGOVINA</b>		No associations	No beekeepers	No hives	Average No hives/beekeeper
		<b>?</b>	<b>?</b>	<b>255755</b>	<b>?</b>

- Data about colony losses and bee mortality still are not accurate and not useful at all!
- There are no official Programs for such specific surveillance identified on whole territory.
- There is National Control Program for some bee disease but data are not powerful tool to be used in the epidemiological control.
- First active survey on CCD or colony *disorders* will be provided with COLOSS questioner during 2009

## Bulgaria

### Diversity, beekeeping and vitality - current situation of Bulgarian bees

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In Bulgaria according to Ruttner’s morphometric analysis (1988) *A. m. macedonica* subspecies occurs. Another point of view is the existence in the country of aboriginal subspecies *A. m. rodopica* (Petrov, 1995). During the period 1950 – 1980, *A. m. ligustica*, *A. m. carnica* and *A. m. caucasica* have been reared too. The main goal of the program for bee selection and queen rearing in Bulgaria is the conservation of the gene fund of the local Bulgarian honeybee. According to the ethological data, the local bees are with high queen fertility, high honey productivity, good winter resistance, low aggressiveness, good hygienic behaviour. The genetic structure of Bulgarian honeybees is studied now through the usage of morpho-ethological, biochemical and molecular-genetic approaches. National Veterinary Service controls the health status of the bee colonies in Bulgaria for: *Varroa destructor*, *Acarapis woodi*, *Nosema apis*, *Bacillus alvei*, *Bac. laterosporus* (Bac. Orpheus), *Melissococcus pluton* (*Streptococcus pluton*), *Enterococcus faecalis* (*Streptococcus apis*), *Peanibacillus larvae*. Till the moment, Bulgaria is free from *Acarapis woodi*. There are no officially data about *Nosema cerana* and IAPV. In the present moment, both pathogens are objects of a joint research supported by Bulgarian Ministry of Agriculture and the results about their presence or absence in the country should be done soon. According to a phone survey with beekeepers from 140 different apiaries (about 13 000 bee colonies) of the National Bee Breeding Association for the period 2006/2007 the colony losses in Bulgaria are about 6% on the average, and for the period 2007/2008 - about 10% totally. About 1% of honeybees in some regions have died because of poisonings with plant protection pesticides. Lack of enough winter nutrient stores, *Varroa destructor*, a tendency of expansion of the areas with rape *Brassica napus*, which could be very dangerous because of pesticide treatment, conduction of plant protection, disinfection and disinsection activities are possible reasons for bee colony losses in Bulgaria during this period. Although, the honey production in 2008 is better than 2007, the beekeepers define this year as a fourth poor year for Bulgarian beekeeping because of meteorological reasons. There are about 750 000 bee colonies in Bulgaria and about 40 000 registered beekeepers at the moment. The National Bee Breeding Association controls the bee colonies from the National gene fund and the Controlled part of the population. Under its control are breeding bases with over than 13 000 bee colonies in total.

## China PR

### Colony losses in China

*Shi Wei*

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China has six million bee colonies and about two hundred thousand beekeepers. Two *Apis* species (the western honeybees, *Apis mellifera*, and the eastern honeybees, *Apis cerana*) are raised in the country; both are bred for bee products (honey, royal jelly, propolis, beeswax) as well as for agricultural pollination. In recent years, beekeepers have been being confronted with several inexplicable and complex symptoms of colony losses on both *Apis* species. Although some of the losses are long known as a result of the damage of *Varroa* mites on *Apis mellifera*, sacbrood viruses on *Apis cerana* and *Tropilaelaps* mites on both species. Other less known factors and mechanisms need to be investigated: 1) Conservation of honeybees *Apis cerana*, *Apis cerana*, the native honeybees in China, is believed tolerant to some pests and pathogens (e.g. *Varroa* mites and American foul brood), now has become a threatened species in China for many reasons, most likely because of competition from the introduced Western honeybee. 2) The common pathogens other than *V. destructor* (e.g. bacteria, fungi and viruses) harm for both species need to be understood, 3) the malnutrition, poisoning and inadequate management effect and bee vitality/diversity on both species request more deeply investment. 4) The mechanisms of *Apis cerana* tolerate the mite, *Varroa destructor*, and the microsporidian, *Nosema ceranae*, must to be contribute. To reach those end, the apicultural situation in China, and for an improved development of beekeeping, the Ministry of Agricultural and the Ministry of Sciences and Technology has granted several actions that are related to the colony losses issue, "Evaluating the negative impact in main honey production regions of China", "Conservation of honeybees *Apis cerana* in China" and the newly established action "Apiculture network on innovation". 20 scientist and 15 research centre have been involved.

## Croatia

### Colony losses in Croatia during winter 2008/09

*Nikola Kezić<sup>1\*</sup>, Maja Drazic<sup>2</sup>, Ivana Tlak Gajger<sup>3</sup> and Zlatko Tomljanovic<sup>4</sup>*

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In Croatia there are no systematic surveys of colony losses so far. Consulting data of Croatian beekeepers federation, there is 4.132 members of this association. Beekeepers range from small hobby operation to large, professional. Colony losses in Croatia 2007/2008 were 27 % estimated by Croatian beekeepers federation. Croatian agricultural agency (CAA) yearly collects data on the number of colonies eligible for state aid in apicultural sector (beekeepers operating more than 30 colonies). During 2007 CAA has registered 3402 beekeepers operating 314943 colonies. Data for 2008 are not processed yet. We have conducted a survey among beekeepers during three beekeepers meetings at the continental part of Croatia during the end of February and beginning of March 2009. In total, 114 beekeepers responded to anonymous questionnaire, with total of 10.293 colonies. In March 2009 questioned beekeepers have 8.716 colonies. During winter 2008/09 period beekeepers lost 1577 colonies (15.32%). Higher losses were reported by beekeepers with experience less than 5 years (25.17 % colony losses) in comparison to more experienced beekeepers (13.50% colony losses). In questioned group 32 beekeepers (28.07%) reported losses over 20%, from which 16 beekeepers (14.03%) reported losses higher than 50% from wintered colonies. At the same time, 13 beekeepers (11.40%) report no losses. From beekeepers opinion, the main reasons were due to problems with queens or food storage (9.10% colonies), *Nosema* and *Varroa* (22.19% lost colonies). From surveyed group, 22 beekeepers did not report causes of loss. Tests on IAPV are not conducted, while *Nosema ceranae* is confirmed in Croatia.

## Denmark

### Colony loss in Denmark

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Focus on colony loss in Denmark has been intensified by the Faculty of Agriculture Sciences and the Danish Beekeepers Association. Severe colony loss has occurred in the past in Denmark. Colony loss is almost exclusively recorded as winter mortality by beekeepers. Since 1986 we have data on colony loss. During this period there were three observations of severe loss. In 1986, before a *Varroa destructor* population established in Denmark, 27 % of colonies died, in 1996, after *Varroa destructor* had spread to most colonies, symptoms similar to colony collapse disorder were reported and 30 % of colonies died, and finally in late fall/spring 2007/08 beekeepers recorded 32,8 % loss of colonies, based on a survey from nearly 6000 colonies. The average winter loss is 16,9 %. However, excluding the three severe episodes in 1986, 1996 and 2008 the average colony loss drops to 12 %, more acceptable to beekeepers. The calculated number of beekeepers in 2006 is 4100. The above figures are from questionnaires in the Danish Beekeeper Associations magazine. Every fifth year a major questionnaire is carried out. In 1986 the number of replies was n = 2.313 (no. of members of Danish Beekeepers Association = 7.332), in 1991 n= 2.185 (members = 6.533), in 1996 n = 1632 (members = 4.907), in 2001 n= 699 (members = 3.734) and in 2006 n = 854 (members = 3.667). For the number of colonies there are no exact data. In the years falling between major questionnaires, mortality has been calculated on the basis of personal interviews to approximately 120 beekeepers selected over the country. The commercial beekeepers of Denmark did a survey amongst 36 of their members based on the high mortality in 2007/2008, reporting losses of 32 % from 11885 colonies. The winter mortality in 2007/08 came suddenly. Losses already started fall 2007 with classical Colony Collapse Disorder symptoms, empty hives with ample supply of food, or just a few bees and the queen left. The same symptoms were reported during much of the winter until the end of April. The explanation for the losses seems to be multi-factorial. Two warm winters in succession, an early spring 2007 allowed *Varroa destructor* to build up strong population in colonies. The number of mites in the dying colonies was much higher than normal (beekeepers are using the term: "mite year"). Due to rainy and cold weather during fall 2007 the pollen supply was poor, reducing the quality of

the winter bees, and the varroa treatment worked unsatisfactory. During the past few years the University of Aarhus included the diagnosis of 7 vira for diseased and dead samples. ABPV and KBV was found in many dead colonies during the winter 2007/2008. Before and after that period these two vira have rarely been found in Denmark. IAPV was not observed in Danish samples. DWV is widespread in Denmark in years of high and low winter mortality. SBV seems more common than in other European countries. *Nosema ceranae* has been in Denmark at least since 2004 and is expected to be widespread; however spore counts does not suggest that *Nosema* is the cause of colony loss. The Danish Beekeepers Association has in 2008 established 10 observations apiaries with one electronic hive scale in each. All the colonies are examined over the season in cooperation with the University of Aarhus. A mite counting group was established reporting mite development over the season. All information will be uploaded direct to the internet, in order to establish an early warning system. *Nosema ceranae* is present in Denmark at least since 2004. IAPV has not been detected as yet in more than a 100 samples from dead colonies in affected apiaries.

Acknowledgement: We are thankful to Keld Brandstrup of EB, for information on losses amongst the commercial beekeepers in Denmark. Financial support was granted from the EU Council Regulation (EC) No 797/2004, to Danish Beekeeper Association and University of Aarhus.

## Egypt

### **Do environment of the new reclaimed land vegetation defeat the symptoms of the honeybee colony collapse disorder (CCD)?**

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Forty five honeybee colonies having symptoms of the CCD were shifted from the Nile village, Egypt, where traditional cultivation and pollution sources, to three locations, fifteen colonies each. Two locations of the selected places were new reclaimed land locations having dried climate, diverse natural flora and no pollution sources; while the third one was a location have the same conditions of the Nile Village. The shifted colonies were treated once with antibiotic (Terramycine) and supplied once with liquid supplement (Aminovite). The colonies placed in the new reclaimed lands showed speed consumption of the supplied food, disappearance of the crawling bees case, significant increase of the daily rate of egg laying, enlarging of the brood area, maximizing the rate of the foraging activity, and extension of the worker longevity. However, the colonies of Nile village location collapsed to a noticeable level. These results may indicate that the clean environment with a diverse vegetation have an important role in defeating the symptoms of the CCD.

Keywords: Honeybees, Environment, Reclaimed land, Vegetation, Colony Collapse Disorder, *Nosema*, Feeding reluctantion, Foraging ,Worker longevity, Terramycine, Aminovite

## Finland

### Colony losses in Finland

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In Finland there are 53000 bee colonies and 2700 beekeepers, of which now 1866 are members. Colony losses have been recorded yearly by Finnish Beekeepers' Association by sending a questionnaire to 10% of members selected randomly. Usually ca. 75% of forms are returned. According to the results, winter loss average from 1998 to 2002 was 16%. A high loss of 34,4% occurred in winter 2002-2003. In 2004-2007 losses were only ca. 10% and in winter 2007-2008 15%. To complement this random sampling questionnaire a new survey to a group of 30 voluntary beekeepers with 3514 bee colonies was established. In this group the losses in winter 2007-2008 were similar to the random survey, 16%. Since the surveys do not reveal the factors responsible to losses, a system to monitor 1-2 apiaries (ca. 10 colonies) of a group of 21 beekeepers by collecting bee samples from them in autumn and spring with colony management details was established. 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 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. We at MTT also continue surveying the prevalence of *N. ceranae* to get more data to determine its role in winter losses by asking beekeepers having *Nosema* symptoms in their hives to send bee samples for analysis.



## France

### Colony losses in France

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In 2004 in France, 69 000 beekeepers owned 1.3 million of hives.

#### **AFSSA case studies:**

From 2002 to 2005, a survey run on 24 apiaries (120 colonies) has shown the multi-exposure of honeybees to low doses of pesticides and the presence of various diseases in colonies, the most serious being varroosis and American foulbrood. During the winter 2005-2006, a study run on 25 apiaries (1503 colonies) that exhibited high losses (66%) had shown inadequate varroa treatments while pesticide residues were not found in dead bees. In 2007, bees from 50 hives (23 sites) were analysed to evaluate CBPV load by Real-Time PCR, following significant high mortality rates. 62% of the surveyed apiaries presented high viral loads, exceeding  $10^{10}$  copies of viral genome per bee, highlighting the major role of CBPV in bee mortalities. To assess the pathological context during the winter 2007-2008, a study was conducted on 35 apiaries (1649 colonies) displaying severe winter losses (68%) in various parts of France. Inadequate varroa treatments were applied in 57% of the apiaries. ABPV and IAPV were detected in 40% and 14 % of the samples, respectively. IAPV was thus detected for the first time in France, not allowing however to establish a causal link with severe winter losses. These data highlight the need for improved molecular detection tools to ensure accurate sensitivity and specificity.

**CNDA survey program:**

A survey was completed to estimate colony losses during winter 2007/2008. 168 professional 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% (IC<sub>95%</sub> = [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 unable to survive. Preliminary results for possible causes show that availability of food, strength of the colonies and varroa pressure could explain partly the losses.

**Varroa tolerant honeybee program**

The stock of varroa surviving colonies is maintained at INRA laboratory in Avignon. The colony survival is checked and the stock is used to find out the causes of the tolerance.

## Germany

### Honeybee colony losses and referring investigations in Germany

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In Germany about 900.000 colonies are kept by about 85.000 beekeepers.

Since 2004 a monitoring project on colony losses is run by the German bee institutes. About 1200 colonies managed by 120 private beekeeping operations are continuously followed over an extended period of time, including at least 3 visits per year by institute staff. Data on various aspects of colony health are recorded, including: colony development, infection data for *Varroa*, *Nosema* and viruses and residue levels of pesticides in samples of bee bread.

So far, the main findings are:

The winter colony losses (between October - March) of the participating beekeepers ranged between 8 and 16 % during the last four years, based on about 7200 colonies wintered by the monitoring partners. Additional enquiries with beekeepers who are not participating in the project resulted in higher losses in this group, thus demonstrating a significant influence of the individual bee keeper.

Significant correlations have been found between winter losses and the infections rates with *Varroa* and the ABPV and DWV rates in the preceding autumn.

A sensitive residue analysis method was established to test bee bread samples for up to 250 active agents. In 215 samples collected during 2005-2007 more than 55 substances were detected, usually in very low levels. Most samples contained several agents, most commonly fungicides, herbicides and acaricides used for *Varroa* control. The residue levels found in the bee bread samples are unlikely to have had a direct toxic effect. However, we are looking to develop suitable experimental setups to estimate the risk of chronic and synergistic sublethal effects on colony health and development.

The monitoring project is complemented by the research activity of single institutes in the fields of *Nosema*, viruses, bee genetics, honey bee immune competence, colony management strategies and related subjects.

For further details on the German monitoring project refer to: <http://www.staff.uni-marburg.de/~ag-biene/dMonitoring.html> (in German)

In spring 2008 about 12500 colonies, mainly situated in the upper Rhine valley were heavily weakened due to Clothianidin exposure. The substance was used as seed dressing in maize. Part of the dressing was loosed during the sowing process and blown into the environment with the air stream of the pneumatic seeders.

## Greece

### Colony losses in Greece:

#### Review of the situation from 2003 to 2009

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Greece has about 22,000 beekeepers holding 1,300,000 colonies. Annual honey production was 16,000 tones for 2006 but much less (almost 50% less) for 2007 and 2008. During winter 2003-2004 many beekeepers were complaining for colony losses higher to 10%. Most complains were in areas where *Nosema* infestations were high and present every year, such as Halkidiki peninsula. In that specific area colony losses at the time exceeded 30%. Later in the same year, DNA identification of *Nosema* spores reveled that *Nosema ceranae* was present in Greece. Colony losses were also reported from other areas during the same period and for the whole year round. Similar estimates were also for the years to follow. A pilot questionnaire survey was contacted in Spring-Summer 2007 and 2008. 166 questionnaires were collected by post during 2007, represented 2% of the total colony numbers (= 26.000 colonies). 385 questionnaires were collected by post in 2008 represented 3,7% of the colonies (= 48.250 colonies). This data showed that winter losses were about 12% for 2006, 15% for 2007 and 14% for 2008. Other losses were also reported due to the use of plant protection products especially on cultivations of cotton, tobacco, corn and oranges. For 2007 these losses are estimated between 3-6%, while about 70% of poisoning cases happen on cotton fields. However, samples arriving in the Hellenic Institute of Apiculture for analysis are mainly from apiaries with high losses and although are treated as individual cases they show clearly the situation in the country. Same way as in 2003-2004, the majority of samples received during 2007 and 2008, showed high infestation levels of *Nosema* disease. The respective apiaries had losses higher than 50%. Most recently, during last months of 2008 and first months of 2009 losses reports were higher with losses reaching the 60% of the colonies in specific areas of North Greece. In an attempt to investigate further the cause of these last years' increasing losses and the interaction of pathogens we started analysis for viruses on bee samples suffering from low and high *Nosema* infestations. Furthermore, a research project on the 'Effects of GMOs, neonicotinoids and air pollution on honey bees' has been granted for 2008-2010 under the Directive 747/04 of E.E.

## Hungary

### First results of the monitoring program on the health status of the Hungarian honeybee colonies

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Apiculture has deep-rooted traditions in Hungary for centuries. More than 15,000 beekeepers work with over 800,000 colonies at present, and the colony density reaches 8-9 per km<sup>2</sup>, one of the highest in the EU. Hungarian beekeepers have been observing with great concern the rise in infectious and parasitic diseases and the lack of reliable disease control. Under the sponsorship of the EU financed National Program of Apiculture coordinated by the Association of Hungarian Beekeepers (OMME), a diagnostic program was initiated in 2007: Samples have been regularly analyzed for nosema, varroa and viral infections; in toto 170 apiaries, with 850 samples, free of charge. Toxicological analyses were carried out on several honeybee and plant samples also, from time to time. The results of the analyses confirmed that mass disorder or mortality with characteristic symptoms, the so-called colony collapse disorder (CCD) is not yet present in Hungary. On the other hand, it was also established that incidences of nosema (particularly *N. ceranae*) and infections of five honeybee viruses (acute bee paralysis virus, chronic bee paralysis virus, black queen cell virus, deformed wing virus and sack brood virus) had become more and more frequent. The feedback from the questionnaires shows that amitraz is the most frequently applied compound in varroa control, although the use of thimol, formic acid and oxalic acid have also been spreading. Pollen shortage due to the dry summer prevented adequate preparation of the colonies for wintering in 2007, thus in some apiaries winter losses reached 30% by the spring of 2008.

## Ireland

### Colony losses in Ireland, a preliminary assessment during 2009/2010

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Ireland is predominantly an agricultural country and thus honeybees are an essential component in maximising crop production and since 75% of flowering plants require bees for pollination they are invaluable for the enhancement of the environment. Since the introduction of the varroa mite (*Varroa destructor*) into Ireland in 1998, many of the feral colonies have disappeared and today there are approximately 2000 beekeepers managing 20000 colonies. Beekeeping in Ireland is predominantly practiced by hobbyists and a total of 60% have <10 colonies, 20-30% have 10-30 colonies and 10% have >30 colonies. Most beekeepers are members of the Federation of Irish Beekeepers and also a member of one of the 46 local associations distributed around the country. As in most European countries, tracheal mite (*Acarapis woodi*), varroa mite (*Varroa destructor*), *Nosema* spp. (*Nosema apis* / *Nosema ceranae*) and foulbroods (*Melissococcus plutonius* / *Paenibacillus larvae*) are endemic to Ireland. Prior to varroa infestation winter colony losses were approximately 10%, but today beekeepers are reporting 15-20%. In 2007, abnormal losses were reported in the West Cork region and results from a questionnaire distributed by the local association reported losses of approximately 53%. Beekeepers' perceived cause of losses included varroa, AFB, starvation and failing queens. Symptoms of CCD were not reported. Although these findings are restricted to a small area and a small number of beekeepers ( $n=98$ ), it is essential to obtain a comprehensible assessment of colony losses throughout the country. Thus, our objective is to carry out the questionnaire on colony mortality produced by COLOSS working group 1 during autumn 2009 / spring 2010.

## Israel

### CCD and honeybee decline in Israel

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Beekeeping contributes about 480 million USD, including the overall impact of due to bee-mediated pollination to Israel's agriculture. The country is considered as one of the most dense beekeeping areas with about 100,000 hives per 7000 km<sup>2</sup>, kept by 450 beekeepers, which produce 3200 metric tons of honey (a value of 12 million USD) and 60,000 cycles of pollination services to different crops, which produce an additional income of 250 million USD.

Recently colony losses increased and were assumed to be about 25%. To evaluate the involvement of various agents, including pathogens, pesticides and beekeeping management, on beehive decline, last November we initiated a comprehensive study of the incidence and characteristics of colony losses in Israel. Major aims of the project are to evaluate: a) symptoms and extent of colony decline and losses, by region; b) the role of pathogens, parasites and pesticides (both applied to field crops and to hives), c) the role of management practices: hive migration, colony nutrition, disease and varroa control. The project consists of: 1. Survey of honeybee colony losses and its potential causes by 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. Developing diagnostic tools for pathogens using sensitive RT-PCR for virus and *Nosema ceranae* detection and dedicated computerized tools for data collection.



Until now, 58 beekeepers (keeping about 46,000 colonies) responded to the survey providing data for 2008. About 40% complained for extensive colony losses. Among the potential causes, the highest damage seems to be associated with workers loss. However, the average losses were about 20%. Some of them observed classical CCD symptoms, however for most of the losses the causes were reported as unknown. So far one round of 113 hives were directly examined and sampled for pests and pathogens towards the end of the winter. In 18.6 % of hives *Nosema cerana* was detected. *Varroa destructor* was detected in 19.4 % of the hives following one hour of Amitraz fumigation. Qualitative 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), Kashmir Bee virus (KBV), Sacbrood virus (SBV) and Varroa derived virus 1 (VDV-1). DWV, VDV-1 and BQV were detected most frequently. Analysis of the relative frequency of these viruses in bee hives is still in process.

## Italy

### Honeybee colony losses in Italy

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Italy accounts for 1,157,133 honeybee colonies (Commission Regulation (EC) No 939/2007 of 7 August 2007) and 75,000 beekeepers. Honeybee colony losses recorded in Italy in winter 2007/08 accounted for 30-40% of the hive population in the Northern part of the country and for 10-30% in the Centre-South, based on oral reports from beekeepers' associations. A survey limited to a few provinces of Emilia-Romagna region (Northern Italy), carried out by means of anonymous questionnaires, showed that of 2,460 colonies managed by 81 beekeepers in summer 2007, 935 (38%) were dead in February 2008. Similarly, an anonymous questionnaire administered to 200 beekeepers of the Veneto region (North-Eastern Italy), showed that 1,299 (37%) of the 3,513 managed colonies were dead in February 2008. The limited availability of data on losses limits a better understanding of the phenomenon and of causative factors. However, the reported losses were mainly attributed to insufficient and/or improper control of varroa infestation and to interactions of *Varroa destructor* with other pathogens, and partially to inadequate apicultural techniques. *Nosema ceranae* was detected in all investigated Italian regions (11 of 20) and added to the list of possible causative factors. Colony Collapse Disorder (CCD), named in USA in 2006, is described as the rapid loss from a colony of its adult bee population, where at the final stages of collapse, the queen is attended only by a few newly emerged adult bees. Collapsed colonies are also described as often having considerable capped brood and food reserves. Despite some of these symptoms have been observed, it seems unlikely that this syndrome has established on the Italian territory. Furthermore, severe weakening or mortality of bee colonies were recorded in spring 2008 (March-June) in Northern Italy. These episodes were associated

with maize sowing procedures. In fact, 57.5% of the 132 analysed dead bee samples collected in this framework were positive to neonicotinoids. A honeybee health monitoring programme on a national basis is in progress, with three ministries possibly involved (Health, Agriculture, and Environment) in order to establish a continuing data collection system. At the same time, a research project has been launched to investigate interactions among different pathogens; the immune response of honeybees to stress agents; the effects of environmental components on welfare and health of honeybees; the effects of agrochemicals on honeybees; the reduction of powder dispersion during sowing of dressed corn seeds; and the synergistic effect of multiple factors on bee health.

## Jordan and Middle East

### **Prevalence of honeybee viruses in the Middle East and their association with the appearance of Colony Collapse Disorder (CCD)**

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Among the pathogens attacking bees, viruses are prime sources of confusion and error in the diagnosis and management of diseases. This is the result of a poor understanding of the dynamics underlying viral disease outbreaks. So far at least 18 honey bee viruses have been reported to infect honey bees worldwide. Moreover, honey bees can be attacked by more than one virus and multiple viral infections have been reported in living bees. Therefore, it is very difficult to identify bee virus infections and almost impossible to differentiate mixed virus infections based only on field observations. Almost certainly this is the first project in the Arab World to suggest using molecular techniques for the detection of bee viruses. At the same time the only laboratory in the Middle East and North Africa conducting basic research in the bee viruses is Professor Sela's laboratory at the Hebrew University of Jerusalem (HUJ). Also the laboratory of the Bee Research Unit (BRU) is the first in the Arab World to use molecular techniques for the detection of the honey bee viruses. Information on the impact of viral diseases in bees in the Middle East will be gathered. Concurrently, a practical means to control viral diseases and the outcome of CCD will also be investigated. Specific objectives are: 1) Identifying virus diseases of bees in the Middle East, 2) Study the integration of viruses into the bee genome and the engendering of virus resistance. 3) Correlation of CCD with bees that have a history of IAPV infection and the association with Varroa infestation. A project vision is to ultimately be able to breed virus resistant bees. The project is granted by the MERC program of the USAID and it started in January 2009.

## Former Yugoslav Republic of Macedonia

### Colony losses in the Republic of Macedonia

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The Macedonian beekeeping sector includes approximately 10.000 beekeepers who own approximately 75.000 honeybee colonies. The annual honey production is about 1.200 metric tones. Neither Macedonian veterinarian service nor other institution in the country does produce any kind of data regarding the beekeeping sector and eventually colony losses rate by year. For that reason, since last COST meeting in Brussels, we have produced questionnaire for assessment of the last winter season (2007/2008) colony losses rate within the Macedonian beekeepers. We have received fulfilled 193 questionnaires back from the same number of beekeepers representing 2% of the total number of the beekeepers in Macedonia. They are located in 12 different regions (cities) across the country. The total number of the colonies of the assessed beekeepers was 11.912 which present 16% of the total population of honey bee colonies in the country. From the analyzed questionnaires, we can conclude that the average colonies losses during the winter 2007/2008 was 20% (2.154 dead from assessed 11.912 colonies). The main 3 reasons for these losses were: lack of food (28%), undefined reason (22%), *Nosema* observation (13%) etc. Related to the mentioned reasons the most "vulnerable" period are early spring (30%), winter (24%) and late autumn (18%). The initial morphometrical analyses show existence and introgression of two races, such as: *Apis mellifera macedonica* and *Apis mellifera carnica*. At the moment, the group of national and international experts is working on molecular determination of the honey bee population in Republic of Macedonia. The results will be published soon.

## Netherlands

### Colony losses in the Netherlands

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Honey bee colony (winter) losses increase in frequency and severity, and much effort is spent in search for the causes. Bees@wur together with the UK Central Science Laboratory tested bee samples in June 2008 of 170 apiaries all around the Netherlands (the Netherlands have about 8000 beekeepers, the median number of hives is 5 per beekeeper) for 8 bee viruses and several fungal, bacterial, microsporidian and acarine parasites. Together with environmental factors and bee management practices these parasites are suspects in the search for the killer. It was found that the number of varroa mites (*Varroa destructor*) was often high in the samples; varroa is a known killer. In 87% of the sampled apiaries the 'novel' microsporidium *Nosema ceranae* was present, whilst the 'ancient' *Nosema apis* only persisted in 10%. Nevertheless there is no indication so far that this ousting, which took place around the world, has caused the increase of colony losses. *Melissococcus pluton*, the cause of European foulbrood, was present in 36% of the sampled apiaries. Prevalence of *Paenibacillus larvae*, the cause of American foulbrood was below 1%. IAPV was not found in any sample. The yearly NCB Dutch monitor on colony losses established a mortality rate (.23) during winter 2007/2008 comparable with the mortality rate during winter 2005/2006 (.26). Data were obtained from (1) 'reference' local beekeeper associations which collected data by telephone (no non respondents), (2) by post and (3) by the internet. The reference data on winter losses by the local organizations were always higher compared with the other sampling methods. The total figures of the monitoring were collected from 812 beekeepers, with 7434 colonies wintered in 2007. 18% of the lost colonies was considered as fitting the USA definition of CCD. We found that CCD losses were increasing in the event that more colonies were managed. Losses in the western part of the Netherlands (m. r. 0.43) differed significantly from the eastern part (m. r. 0.21). Varroa management was not different in both regions. This is interpreted as indication that besides varroa other causative factors play a role. On the island Texel the dispersal of *Nosema ceranae* was studied by NCB. The results support the hypothesis that *Nosema ceranae* arrived in 2007

vectored by the import of contaminated beekeeper material. In 2008 a rapid spread of *Nosema ceranae* over the island was observed. In May 2008 32% of the colonies tested positive. In the NCB case cohort study (N= 300 colonies), started in 2008, a significant correlation was found between the mortality rate per apiary in the winter 2007/2008 and the percentage of remaining colonies found positive for *Nosema ceranae* in May, 2008. In 4 subgroups of tested colonies (3 islands and the mainland) we could establish a significant negative correlation between the exposure on *Nosema ceranae* and *Nosema apis*. More *Nosema ceranae* resulted in less *Nosema apis* and less colonies with both *Nosema* sps. Our *Nosema* sps. research is performed in collaboration with the Spanish Centro Apicola Regional (CAR).

## Norway

### Colony losses in Norway

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Due to the heavy losses of honeybee colonies reported in USA and several European countries the beekeepers in Norway are worried that they will be facing the same problems, including the phenomenon Colony Collapse Disorder (CCD). Due to its location and restrictive import regulations for honeybees, Norway is among very few (if any) countries with European honeybees where the ectoparasitic mite *Varroa destructor* is still not established throughout the country. There are about 3000 beekeepers and 60 000 honeybee colonies in Norway. As a part of the monitoring program for colony losses in Norway a questionnaire was sent to the 2700 members of the Norwegian Beekeepers Association. We received 524 questionnaires (19%). These beekeepers lost 1813 of their 17 872 colonies from wintering in 2007 to the end of season in 2008. This 10% rate of colony losses is in the same range as in previous years and not unexpectedly high in a country with a harsh climate. *Nosema ceranae* was identified for the first time in 2007 both in mixed infection with *Nosema apis* and as single infection in one sample. The presence of bee viruses other than DWV is unknown as no virological analyses have been made. The rate of colony loss in the parts of the country where varroa is not present was significantly lower (6.6%) than where the presence of varroa has been verified (11.3%,  $P < 0.001$ ). As varroa seems to be a key factor involved in CCD the situation in Norway might represent unique, but so far, unexplored possibilities for epidemiological studies and studies of pathogen interactions.



## Poland

### Colony losses in Poland in the winter of 2007/2008

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There are approximately 1 million honeybee colonies and 40000 beekeepers in Poland. The analysis of questionnaires sent to Warsaw University of Life Sciences by 426 beekeepers, concerning 26710 bee colonies in Poland, indicated that in our country colony loss during the winter of 2007/2008 (15.3%) was significantly higher than during the winter of 2006/2007 (9.9%). The mean colony loss for an apiary was 15.9%. The highest percentage of beekeepers (22.9%) who lost 30(+) percent of their colonies was within the group of owners of 21 to 50 colonies. Among 7 provinces, from which we received enough data for statistic analysis, the losses were highest in Zachodniopomorskie – 30% and Wielkopolskie – 25% dead colonies. However, according to the estimation of beekeeping associations (phone surveys) in Poland during the 2007/2008 season the total colony loss was about 30%. This means 300 000 dead bee colonies. Annual total costs resulting from the winter loss, depending on estimated percentage (ca 15 or 30%), were respectively 48 000 000 or 96 000 000 € (total of: colonies losses, reduced honey, wax and pollen yield, as well as lost pollination value of agricultural crops). The investigation of 448 dead bee samples and 15 capped brood samples (from collapsed or almost collapsed colonies) sent by 104 beekeepers from October 2007 to April 2008 revealed a “Varroa problem” (severe *Varroa destructor* infestation, presence of bees with deformed wings or bees heavily infected with acute bee paralysis virus - ABPV) in 55% of the apiaries. In 32% of the apiaries a severe *Nosema* spp. infection was detected. In almost 50% of the latter heavy infection with black queen cell virus (BQCV) was present. We were not able to determine the possible causes of colony losses in about one third of the apiaries. The investigation of the 1191 dead bee samples sent to the National Veterinary Institute, by 300 beekeepers,

who lost 30(+) percent of their colonies, revealed that 62% of apiaries from which the samples were sent had a problem with a severe *Varroa* infestation and about 55% had a severe *Nosema* spp. infection. From among investigated viruses CBPV was found in 8% samples of dead bees, ABPV in 37% and DWV in 79% samples. This winter (2008/2009), no heavy colony losses have been observed, up to the first days of February, however, beekeepers noticed an unusual dwindling of the colonies. Many of these colonies will probably have died by spring.

## Portugal

### Honey bee diseases and colony losses in Portugal:

#### Results from the last nationwide survey

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In many countries, beekeepers are reporting abnormally high honey bee colony mortalities. Portugal is no exception in this matter. Despite the fact that beekeepers' reports on colony losses have recurrently been made, it seems that colony losses are occurring more frequently and to a larger extent nowadays. Portugal has approximately 15.000 registered beekeepers that keep  $\pm$  550.000 colonies in circa 33.000 apiaries. The last nationwide survey on honey bee diseases was carried out in 2006, focusing on 359 apiaries owned by different beekeepers spread throughout the country. Capped worker brood and adult honey bee samples were collected. Beekeepers were also asked to fill in a questionnaire covering a number of honey bee colony sanitary issues. All the samples were analysed by the Portuguese Laboratory for Veterinary Research (national reference lab for bee diseases), leading to the following results. *Varroa destructor* was found in samples representing 27.0% of the studied apiaries; *Senotainia tricuspis* larvae were identified in 19.8% of the adult bee samples; *Nosema apis* was present in 18.8% of the adult bees samples; *Ascosphaera apis* infested mummies were found in 3.7% of the brood samples; *Malpigamoeba mellificae* was recognized in 2.3% of the adult bee samples and *Paenibacillus larvae* was detected in 2.3% of the brood samples. *Acarapis woodyi* was only observed in 0.3% of the adult bee samples. Honey bee viruses, malnutrition or poisoning, as well as management suitability in a context of climatic changes, were not assessed. Concerning the magnitude of annual colony mortality, an average value of 30.3% was reported. When asked about the reasons for such colony losses, 78.0% of the enquired beekeepers were unable to associate it with any specific cause, 15.0% blamed it on *Varroa* and 4% on American fool brood. Regarding seasonal episodes of colony mortality, winter was clearly the most critical season (53.5% of the enquired beekeepers reported colony losses overwinter), followed by autumn (17.3%), summer (10.9%) and spring (7.9%). Despite the fact that *Varroa* seems to keep playing a key role in colony mortality in Portugal, it does not appear to fully explain contemporary losses, therefore creating a need to better ascertain honey bee colony death origins in Portugal. It is anticipated that COLOSS will be instrumental in this respect, as well as in moving closer to a set of reliable mitigating measures to counteract this alarm causing situation.

## Serbia

### Honeybee Colony Losses in Serbia

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In Serbia at the moment, there are approximately 20000 beekeepers with about 400000 honeybee colonies. Only one third of all beekeepers are members of Serbian Beekeepers Association. It was estimate that bee colony losses for the overwintering period 2007/2008 were about 110000 colonies (27.5%), mainly according to personal communications. It was reported numerous causes contributing to the colony losses in 2008 mostly received by phone or personal communications. Also, some relevant data recently obtained from Serbian Beekeepers Association for overwintering period 2008/2009 (to the end of February 2009), approximate to 30% colony losses. In addition, according to the decision of the Ministry of Agriculture, Forestry and Water Management of Serbia and the Department for Plant Protection of the Republic of Serbia, a professional Committee was formed in the beginning of July 2008 with the assignment to establish the cause of bee losses on the field in the period July 10 to 15, 2008 (when bee colonies were on a sunflower pasture) on 14 apiaries all over Serbia. In all estimated apiaries there were 1640 honeybee colonies. The following data were considered in each of the apiaries: the strength of the bee colonies, the number of dead bees in front of the bee hive, on the floor and the quantity of bees in the bee hives, the chemicals used to control *Varroa destructor* mite, the behaviour of bees in front of the beehives. No presence of American foulbrood, occasional presence of varroa and low presence of *Nosema* spp. was established on any of the apiaries (average invasion has been established in 4 apiaries and low invasion in 5 of them). In one apiary, high bee loss was determined, in 8 apiaries the loss of bees was low and there were four apiaries with dead bees in front of the bee hives. No presence of dead bees was established on the floors of the bee hives. The chemical analyses have not been finished yet, but the mortality of bees in Serbia so far appears to be related to the pollen in the beehive coming from the sunflower pasture. In Serbia to the present time not found IAPV virus. *Nosema ceranae* was detected in Serbia from 2006.

## Slovakia

### Slovakian honey bee colony-loss in the season 2007/2008

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In the year 2007 totally 247 678 bee colonies was registered in Slovakia, in the following year 2008 decrease to 219 793 colonies was recorded. Inter-annual decrease was thus app. 11.25%. The losses were detected mainly in smaller hobby apiaries with less than 20 colonies. The main causes of these losses are most probably *Varroa destructor* and *Nosema* spp. infection together with associated viral infections and incorrect timing of treatment by some beekeepers. Methodology of treatment of bee colonies against diseases should be modified every year depending on wheather and climatic changes. Occurence of following pathogens is regularly monitored by veterinary bodies: *Paenibacillus larvae*, *Varroa destructor*, *Nosema* spp., *Aethina tumida*, viruses DWV, SBV, CBPV, ABPV, KBV, BQCV. In 2007 were analysed 53 of bee samples with following positive findings: 2 (3.5%) DWV, 25 (43.8%) ABPV, 3 (5.3%) SBV, 1 (1.75%) KBV. In 2008 were analyzed 31 samples with positive findings as follows: 14 (45.1%) ABPV, 9 (29%) SBV. In 2008 first occurrence of *Nosema ceranae* on one locality was detected as well.

## Slovenia

### Colony losses in Slovenia

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The Slovenian beekeeping industry includes approximately 8000 beekeepers who managed about 170.000 honeybee colonies. The annual honey production is about 2000 metric tones. The average No. of populated hive per square km is at the highest level in EU (8.5 colonies/km<sup>2</sup>). Colony losses due to winter mortality have been reported regularly since 20 years, but the last year we recorded unusual high losses of bee colonies. The rough unofficial estimates for the period from fall 2007 to spring 2008 ranged from 30 to 50% of colonies. The small surveys by personal contact including 57 apiaries showed an average loss of 32±0.15% with the range from 0 to 100% loss of colonies for the same period. The survey did not indicate any correlation with the presence of agricultural activity, but the survival of colonies was linked to the use of acaracides against the parasitic mite *Varroa destructor*, which might indicate that the successful mite control was a key factor for survival. However, data from the Sector for Registration and Identification of animals (SIR) including 7737 successfully completed registrations for apiaries do not show any decline in bee population as data were collected in fall 2007 and fall 2008. This indicates that beekeepers have recovered winter losses and re-established colony fund. We think that the main factors for unusual high colony losses are *Varroa destructor* accompanied with secondary infections (viruses), Nosemosis and environmental and other influences (pesticides, insufficient nutrition and technological deficiency in beekeeping).

## Spain

### Colony losses in Spain

*Higes M., Meana A. and Hernández R.*

No official data about colony losses in Spain are available although they are estimated between 20-40%, based mainly in oral denounces. The higher number of losses usually is detected on autumn and after wintering and no any regional pattern on losses has been detected. A high prevalence of pathogens as *Varroa destructor* and *Nosema* sp. are the main responsible of losses. Pesticides can be a problem in some specific areas.

## Sweden

### Colony losses in Sweden

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The number of beekeepers and colonies is about 12000 and 125000 respectively. Data on colony losses have for many years been based on the yearly reports from the beekeepers to the Swedish Beekeepers Association (SBR). 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 were 12 % of 33800 wintered colonies. The losses 2007/2008 were 17 % of 31400 wintered colonies. The figures are based on reports from 4296 respectively 3714 beekeepers (which is about 44 % and 41 % respectively of the members of SBR). So far we have no figures on losses from the winter 2008/2009 and data based on the reports to SBR will not be available until March 2010. Because reports of exceptional colony losses the winter 2007/2008 in a county in central Sweden, a thorough survey (phone interviews) was carried out in that county in the spring of 2008. We received answers from 234 members in that county (a little more than 80% of the members having bees). Out of 3619 wintered colonies 1303 died, a loss of 36%. The losses were higher in apiaries with Varroa than in apiaries without Varroa. The more efficient Varroa had been controlled the lower were the losses. In apiaries where no or inadequate control was conducted, losses were very high, about 70% in total. Although the collected information is based on questionnaire rather than on hard data, the overall pattern is undoubtedly that the major factor causing colony losses in the area studied is inadequate Varroa mite control. The aim is to develop a system to continuously collect reliable information where feedback to the beekeepers can be faster. Different methods to accomplish this objective are under evaluation. In addition to collecting information on winter losses we are working on establishing a number of study apiaries where data and samples are continuously collected. Inadequate Varroa control appears to be the main reason for colony losses in Sweden. Nevertheless we are also pursuing studies to evaluate if changes in nosema parasite species composition reported in the literature will influence the losses of colonies over time. Furthermore, we also study the composition of virus infections and how virus infections interact with other pathogens to understand how virus infections contribute to colony losses.



## Switzerland

### Colony losses in Switzerland

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In Switzerland, beekeeping is with very few exceptions in principal a hobby and about 18'000 beekeepers manage ~190'000 bee colonies. Since the winter 2002/03, Swiss beekeepers appear to suffer regularly from unusually high colony losses. To obtain a more detailed picture of the losses and identify possible causes, we conducted a survey in spring 2003 throughout Switzerland. A questionnaire was included in the Swiss beekeeping journal resulting in 557 useable answers (~4 % of the Swiss beekeepers). The mean colony losses 2002/2003 were 23% and 12% of the beekeepers lost much than 60% of their colonies. We found no significant influences of geographic pattern, apiary altitude, crop (maize, rape and sunflower) and *Varroa destructor* control management. However, such a questionnaire is probably not sensitive enough to evaluate the whole complex Varroa control picture (number and timing of treatments, type of product used, ambient temperature during treatments, quantity of product, precise mode of application, re-invasion, etc.). In Winter 2003/04, losses were considered being normal (<10%). Based on rough estimations, mean losses in winter 2004/05, were 10-15%, but regionally peaking up to 30%. In winter 2005/06, losses were about 30% according to regional (canton) monitoring systems. Based on a non-systematic survey about colony losses in the German speaking part of Switzerland less than 10% of colonies were lost in winter 2006/07, which is considered being normal. In Winter 2007/08, we got the first results from the newly established national monitoring system which was implemented in 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. From the 1<sup>st</sup> of October 2007 till the 1<sup>st</sup> of April 2008, the mean colony losses in Switzerland were 18% (ranging from 5-35% depending on the canton). 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. Up to now, no local IAPV analyses have been performed. We can't exclude that pesticides used in agriculture or in apiculture are also involved. Several ongoing projects at our Centre are aiming to identify the drivers for honeybee colony losses.

## Turkey

### Colony Losses of Honeybees and the New Structure of Beekeeping in Turkey

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Beekeeping is important for Turkish economy. In 2008, the important development for Turkish beekeeping is revision of Beekeeper Registration System. Ministry of Agriculture and Turkish Beekeepers Association started the use of a barcode system for registration from hive to honey jar. Presently 33.770 beekeepers with 3.300.000 colonies were registered. Coincident with Colony Collapse Disorder (CCD) news in the US, extraordinary colony losses between 2006-2007 have been reported from several eastern provinces in Turkey. In 2007, we have conducted a survey study on a sample of beekeepers from around Turkey, 288 questionnaires obtained by email, personal visits or by beekeepers associations of provinces representing over 35.000 colonies. The analysis indicated 30% overall colony loss. In contrast, in 2007-2008 winter only Thrace region reported higher than usual losses and this is under investigation. Surprisingly, from all regions of Turkey, 2008 winter losses were 1.8 % or 59.400 colonies as assessed by a simple questionnaire. Considering the presence of over 4 million colonies in Turkey, 1.8% is a large number. Notwithstanding, we evaluate 2008 losses as "normal winter loss". Among the causes for losses, *Varroa* was the biggest problem. This raised concern on viral infections. In addition to *Varroa*, viruses and *Nosema*, we found other problems such as pesticides, starvation, incorrect disease treatment, old queens, comb foundation quality, climatic change etc. For 2008 winter we focused on climatic changes, since these could augment all other factors. The new barcode system can be used for bee health monitoring, for following routes of migratory beekeepers and spread of diseases.

## United Kingdom

### Honey bee colony losses in the United Kingdom

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Note from April 2009 the CSL will become part of the Food and Environment Research Agency (Fera)

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Honey bees are vital pollinators in commercial crop production (estimated UK value £200 m.p.a.), and also in natural ecosystems, where their role in maintaining biodiversity is almost incalculable. The value of UK honey production varies between £10 to £35 m.p.a. It is estimated that there are 230,000 colonies in England, managed by ~30,000 beekeepers, of which 99% are hobbyist, and only 1% commercial enterprise; Wales has ~20,000 colonies and 4,000 beekeepers; Scotland has ~36,000 colonies and 6,000 beekeepers; in Northern Ireland 4,000 colonies are managed by 1,000 beekeepers. Recently, beekeepers across North America and Europe have reported increased and sudden losses of colonies. The term colony collapse disorder (CCD) has been used to describe this phenomenon in the US. However, the specific symptoms associated with CCD have not been described in the UK where, historically, annual colony losses have fluctuated greatly. Since 2001, when pyrethroid resistant *Varroa* mites were first detected, there has been a gradual increase in colony losses in the UK. In 2008, Winter/Spring losses for England and Wales, based on the results of a survey of 1,385 beekeepers owning 10,897 colonies, showed over-winter losses of 33% for 2008. Total losses throughout 2008 were 12% (26,463 colonies inspected). Over-winter losses in Scotland for 2008 based on a Scottish Beekeeping Association written survey of 10% of its membership were 27%. Anecdotal evidence for Northern Ireland suggests that colony losses for the same period were >20%. The major impact on colony survival across the UK has been the *Varroa* mite, but other pathogens, pesticides, bee nutrition and colony management by the beekeeper have also been implicated. It is likely that the increased honey bee mortality may not be due to a single factor, but to multi-factorial interactions between these various stressors. In England and Wales a Bee Health Strategy has been developed by The Department for Environment and Rural Affairs (Defra) and The Welsh Assembly Government (WAG), in conjunction with key industry Stakeholders. This sets out a plan for the future direction of work aimed at sustaining the health of honey bees and beekeeping in England and Wales for the next

decade (Parallel programmes are being considered in Scotland and Northern Ireland). It sets out five key outcomes: 1) Effective communications and relationships operate at all levels; 2) Effective biosecurity minimises risks from pests, diseases and undesirable species; 3) Good standards of beekeeping and husbandry minimise pest and disease risks and contribute to sustaining honey bee populations – prevention is better than cure; 4) Impacts from pests, diseases and other hazards are kept to the lowest levels practicable; and, 5) Sound science and evidence underpins bee health policy and its implementation. The strategy proposes objectives and activities that will contribute to achieving these desired outcomes. The National Bee Unit will be instrumental in the implementation of the strategy. Information gathered through the work of the strategy can be used to feed into the European COLOSS working group.

## United States of America

### Colony losses in the United States of America

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The health of honey bee colonies in the U.S. seems to be improving although CCD occurrences continue. The Apiary Inspectors of America and USDA-ARS estimate that honey bee colony losses for fall/winter 2006-2007 and 2007-2008 were 31% and 36% respectively. These loss estimates are based on phone surveys of beekeepers who manage between 10-18% of the 2.4 million colonies in the U.S. Numerous causes, including CCD, were reported as contributing to the colony losses in both 2007 and 2008. Anecdotal evidence suggests that colony losses have been lower during the 2008-2009 fall/winter season. The next U.S.-wide survey of colony losses will be conducted in late March/early April 2009 to substantiate the anecdotal reports. The cause(s) of CCD in U.S. bee colonies remains under investigation. Currently, many conceivable and realistic hypotheses remain plausible. Not listed in any particular order, these hypotheses include, but are not limited to: (1) traditional bee pests and diseases, (2) how the bees were managed, (3) queen source (poor genetic biodiversity), (4) chemical use in bee colonies, (5) chemical toxins in the environment, (6) varroa mites and associated pathogens, (7) bee nutritional fitness, (8) undiscovered/newly-discovered pests and pathogens, and (9) potential synergistic interactions between two or more of the hypotheses. Other hypotheses have been proposed (genetically modified crops, climate change, etc.) but those listed above currently are the most investigated.

## The COLOSS puzzle: filling in the gaps

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In preparation for the 4<sup>th</sup> COLOSS Conference in Zagreb, 32 abstracts from 30 participating countries were submitted. Most of them reported dimensions of potential colony losses and status of monitoring originating from different surveys or updated the situation regarding parasites and pathogens in the particular country. To obtain a standardized and uniform up-to-date overview on colony losses in the COLOSS participating countries we summarized what the participants have submitted. No other literature than the submitted abstracts were used. The number of beekeepers and colonies in the participating COLOSS countries was 566,818 (excl. China and the US) and 15,620,543 (excluding Netherlands and Denmark) respectively. The latter is almost twice as high as earlier estimates of the number of colonies kept by beekeepers in Europe (~8 Mio). In total 611,767 colonies were included in the reported surveys over several years. While some countries already have well-established surveillance programmes (apiary inspectors, USA; DEBIMO, Germany), hard field data are still lacking or pilot surveys have just started in other countries. In our summary (Table 1), we included data about the number of beekeepers and managed colonies, type of survey and/or name of surveillance programme and total or average losses of colonies in percent, where available. Please refer to the respective abstracts for details. As a parameter of validity we included the number of checked colonies in fall (sample size) whenever possible. We also asked participants to report the presence of two current suspects for causing CCD (iAPV and *Nosema ceranae*). At least four countries (Germany, Greece, Italy and Serbia) also reported additional colony losses or weakenings later in the year, presumably due to plant protection products. In light of the submitted abstracts, we conclude that more extensive and internationally standardised surveys are urgently required, which will form the basis for future hypothesis-driven research on the causes of colony losses.

**Table 1:** Overview of colony losses in the participating countries based on data presented in the submitted abstracts. The number of beekeepers and colonies, year of survey, colony losses [%], N = sample size of checked colonies, type of survey, presence of *Nosema ceranae* and iAPV (unknown=?) and references are given (author and page number in the Proceedings of the 4<sup>th</sup> COLOSS Conference).

Country	Beekeepers	Colonies	Years	Losses [%]	N	Type of survey	<i>N. ceranae</i>	iAPV	Author(s), page(s)
Austria	22198	278810	2007/08	13.3	16217	questionnaire	YES	NO	Crailsheim et al, p. 7
Belgium	8600	101600	2004/05	16.4		questionnaire, visits, sampling	?	?	Nguyen et al. , p. 8
			2006/07	7.3					
Bulgaria	40000	750000	2006/07	6	13000	phone survey	?	?	Ivanova & Petrov, p. 10
			2007/08	10					
Croatia			2008/09	15.3	10293	questionnaire	YES	?	Kezić et al. , p. 12
Denmark	4100		2007/08	32	17000	questionnaire	YES	NO	Vejsnæs & Kryger, p. 13
Finland	2700	53000	2007/08	16	3514	voluntary survey	YES	NO	Korpela et al. , p. 16
				15	2826	random survey			
France	69000	1300000	2007/08	29.3	62400	CNDA survey	YES	YES	Chauzat et al. , p. 17
Germany	85000	900000	2004ff	8-16	7200	DEBIMO	YES	YES	Büchler et al. , p. 19
Greece	22000	1300000	2006/07	15	26000	questionnaire	YES	?	Hatjina et al. , p. 21
			2007/08	14	48250				
Hungary	15000	800000	2007/08	10-30	170	Diagnostic program	YES	NO	Békési & Mátray, p. 22
Ireland	2000	20000	2006/07	53	891	questionnaire	YES		Coffey & Breen, p. 23
			2007/08	15-20		unofficial estimates			
Israel	450	100000	2008/09	20	46000	questionnaire	YES	YES	Soroker et al. , p. 24
Italy	75000	1157133	2007/08	37.4	5973	questionnaire	YES	NO	Mutinelli et al. , p. 26
Former Yugoslav Republic of Macedonia	10000	75000	2007/08	18	11912	questionnaire	NO	NO	Uzunov et al. , p. 29
Netherlands	8000		2007/08	23	7434	NCB Dutch monitor	YES	NO	Blacquièrè & van der Zee, p. 30
Norway	3000	60000	2007/08	10.1	17872	questionnaire	YES	NO	Dahle, p. 32
Poland	40000	1000000	2007/08	15.3	26710	questionnaire	YES	?	Topolska et al, p. 33
Portugal	15000	550000	2006	30.3		national survey	?	?	Murilhas, p. 35
Serbia	20000	400000	2007/08	27,5		estimation	YES	NO	Mladenović et al., p. 36
Slovenia	8000	170000	2007/08	30-50		estimation of total population	YES	?	Gregorc & Kralj, p. 38
Sweden	12000	125000	2006/07	12	33800	questionnaire	YES	?	Kristiansen & Fries, p. 40
			2007/08	17	31400				
Switzerland	18000	190000	2002/03	23	8200	questionnaire	YES	?	Charrière & Neumann, p. 41
			2007/08	18		monitoring system			
Turkey	33770	3300000	2006/07	30	35000	survey	YES	?	Özkırım et al., p. 42
			2007/08	1.8		questionnaire			
United Kingdom	41000	290000	2007/08	33	10897	questionnaire	YES	NO	Wilkins et al. , p. 43
USA		2400000	2006/07	31		Apiary Inspectors America/USDA-ARS	YES	YES	Ellis et al. , p. 45
			2007/08	36					

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