



Action FA0803



COLOSS WORK SHOP

WG1

STANDARDS ON MONITORING & POSITIVE FEED-BACK LOOPS BETWEEN SCIENTISTS AND
BEEKEEPERS

14TH TO 16TH JUNE 2010, ANKERHUS, SLAGELSEVEJ 7A, DK-4180 SORØ, DENMARK

&

WG4

STANDARDIZED PROTOCOLS FOR HONEY BEE VITALITY AND DIVERSITY

16TH TO 18TH JUNE 2010, AARHUS UNIVERSITY, FACULTY OF AGRICULTURAL SCIENCES,
DEPARTMENT OF INTEGRATED PEST MANAGEMENT, RESEARCH CENTRE FLAKKEBJERG, DK-4200
SLAGELSE, DENMARK



ABSTRACTS

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WG4

Standardized protocols for honey bee vitality and diversity

16th to 18th June 2010, Aarhus University, Faculty of Agricultural Sciences, Department of
Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse, Denmark

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WG1

Standards on monitoring & positive feed-back loops between scientists and beekeepers

Estimation of honey bee colony losses within professional beekeepers in France during the winter 2008/2009

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This work is presented by Dr. Yves Le Conte (INRA) at the COLOSS Working Group 1 session in Danemark (June 14-16/2010)

During recent years, professional French beekeepers have found an increase in winter honey bee colony losses (mortality, weakness, diseased or queenlessness). A lot of causes have been mentioned to try to understand those losses, like phytosanitary treatments, parasites, diseases, lack of biodiversity in farmlands, but it has been impossible to clearly make any conclusions.

In 2007, the French technical and scientific institute for beekeeping and pollination (L'ITSAP-Institut Scientifique de l'Abeille et de la Pollinisation) launched a survey with the aim of improving general knowledge about winter bee losses. The questionnaire contained questions about beekeeping practices for wintering preparation, colony background during the season, environment of apiary.

This first year was in fact a pre-study, 168 professional french beekeepers (more than 150 hives) were randomly selected out of 782 beekeeping farms. The losses rate was 29% (IC95% = [26% - 32%]) at the national level, and the following results have been obtained with this first survey:

- Estimate mean of colony losses, regional and national.
- Elaboration of a methodology.
- A typology of apiary related to loss rates.
- Identification of risk factors, and interactions between those factors.

In 2008, a second sample group was defined and the questionnaire was filled in. Preliminary results for possible causes show a correlation between availability of food, strength of the colonies and varroa pressure with the losses. During the winter 2008/2009, the losses rate was 23% (IC95% = [21% - 25%]).

A deep study of those data has been done, with the aim of confirming the typology, the risk factors and their interactions, and of developing prediction models of the loss rates.

We intend to extend this national survey over several years to get a close monitoring of loss rate according to the COLOSS basic questionnaire.

We will be able, next September 2010 at the COLOSS WG1 meeting, to show:

- the results of the different statistics methods we used to better understand the links between beekeeping practices before and during wintering in France and the colony losses in the next spring.
- the first figures on bee colony losses during the winter 2009/2010.

The survey combining the COLOSS questionnaire and the questionnaire prepared for colony losses in Turkey

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Turkey is enjoying very high genetic diversity of honey bees such that about twenty per cent of the honey bee races in the world could be found in this country. We intended to study the genetic basis of vitality of the different races.

We have chosen five regions representing most of this genetic diversity to carry out the survey on honey bee losses where we used a single questionnaire combining the COLOSS questionnaire and the questionnaire prepared to study the colony losses in Turkey. These five regions are also known to have both migratory and local colonies. We surveyed both migratory and local beekeepers to investigate the effects of migratory beekeeping on honey bee losses. The surveys are carried out both by face to face interview or by distributing the questionnaire to the beekeepers and collecting them later. Although the survey is continuing, our preliminary results for the three regions were as follows: 1. In the Mugla province which is one of the most important centres for beekeeping especially for pine honey production, proportion of the colony losses was 0.175 ± 0.035 when a sample of 41 beekeepers interviewed. 2. In the Hatay province where *Apis mellifera syriaca* exists locally, the proportion of colony losses was 0.196 ± 0.032 in a sample of 32 beekeepers 3. In the Trace region, which includes the Canakkale, Tekirdag, Edirne, Kirklareli and Istanbul provinces colony losses averaged 0.193 ± 0.025 in a sample of 59 beekeepers. Overall average of these three regions is 0.187 ± 0.017 . We still have to study 4. The Ankara province and 5. The Eastern Blacksea region which includes the Artvin, Ardahan, Rize and other provinces.

These results involve only face to face interviews and surveys done indirectly are not included at this stage. The results involving indirect surveys will be included at a later stage as they come.

Colony losses in Sweden 2009-2010

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From 29th of April to 30th of May we have conducted a web-based survey on colony losses 2009-2010. The questionnaire is based on the basic COLOSS questionnaire that was the result of the meeting in Amsterdam in January 2010.

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

Winter bee losses data collection in Italy

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Data collection on honey bee losses has attracted the attention of beekeepers, researchers as well as of health authorities in different countries during recent years. Italy accounts for 1,157,000 honey bee colonies (Commission Regulation (EC) No 939/2007) and approximately 75,000 beekeepers. Reports of losses from Italian beekeepers lead to two main scenarios: spring and summer losses, mostly caused by incorrect use or abuse of pesticides; late summer and winter losses caused by *Varroa destructor* and associated diseases. Based on the Italian monitoring network "APENET", 2009/2010 winter losses amounted to 17.6% (113 dead hives/753 hives). Higher losses were recorded through the COST questionnaire applied in the Veneto region (northeastern Italy) with approximately 56,000 hives officially registered. 153 returned questionnaires representative of 4,994 beehives revealed a 2009/10 winter mortality of 23%. The same questionnaire was applied by telephone calls in the Lazio region (central Italy) indicating a winter colony loss of 15.7% (38 responders with 7,939 hives). Winter mortality recorded in Italy in 2007/2008 and 2008/2009 amounted on average to 37% and 11 to 24%, respectively. The main cause reported by beekeepers to explain winter bee losses was the difficulty encountered to carry out an appropriate control of Varroa mite infestation. This was mainly linked to the limited effectiveness of thymol-based medicines and, in general, to the limited availability of medicines for the control of varroaosis. The questionnaire seems able to provide relevant information on colony losses despite its application on the national territory still being very limited. Furthermore, beekeepers and their associations need to be sensitized to an active participation for an appropriate collection of information.

The application of basic coloss questionnaire in Turkey

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In this study, The Basic Coloss Questionnaire were applied to Turkish beekeepers from different regions in Turkey. 3500 Turkish beekeepers joined the study to answer the questions in the questionnaire. The questionnaire were applied to the Turkish beekeepers in 8 different cities in Turkey by only handwriting in the beekeeping seminars. The Turkish Central Beekeepers Association sent the questionnaire form to the City Beekeepers Associations by e-mail in 81 cities. The essential and optional questions were asked to the beekeepers. All data were collected and the answers classified. All data were analysed with basic statistical analysis. During the study, it was recognised that there is a correlation between the length of the winter period and the death of the colonies. Another correlation is between the use of different races of bees and the death of colonies. The study is not completed yet. When the all data are collected from the beekeepers from 81 cities in Turkey, all data will be analysed with a standard statistical method which is decided by WG1 and evaluated again by considering other alternatives.

Preliminary results of this year's winter losses in Poland

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In Poland the survey on colony losses during the winter of 2009/2010 was very seriously upset by two tragedies: in April - the crash of the president's plane which killed 96 people including many of the most important people in our country and enormous floods in May with which many people are still struggling. Until May 29, we received questionnaires from 202 beekeepers (with 8871 colonies) as a reply to an announcement published in the beekeeping journal "Pszczelarstwo", 86 letters posted and e-mailed to different beekeeper associations, 130 e-mails sent to individual beekeepers and a presentation during two beekeepers meetings. Most data concern the losses in Wielkopolskie where the beekeepers managed to fill in a questionnaire during the meeting held on the day of the crash but before the first news of the tragedy appeared in the media.

An analysis of these partial data showed that the colony losses last winter were about 17%; however, in Wielkopolskie the losses were about 13%, while during the previous year they were one of the highest in Poland and reached about 18%. 9% of colonies died from CDS (colony Depopulation Syndrom). Beekeepers attributed their losses to varroosis (20%), weak colonies in autumn (14%), nose mosis (13%), poor queens (12%) and starvation (9%). The survey was done using the COLOSS questionnaire, also on the website www.beemonitoring.org (courtesy of Romee van der Zee).

Preliminary Results: Honey bee colonies losses in the U.S., winter 2009-2010

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The Apiary Inspectors of America (AIA) and USDA-ARS Beltsville Honey Bee Lab conducted a survey to estimate winter colony losses for 2009/2010. Over 22.4% of the country's estimated 2.46 million colonies were surveyed.

A total loss of 33.8% of managed honey bee colonies was recorded. This compares to total losses of 29%, 35.8% and 31.8% recorded respectively in the winters of 2008/2009, 2007/2008 and 2006/2007.

In all 4,207 beekeepers responded to the on-line survey and an additional 24 were contacted by phone. This response rate is orders of magnitude greater than previous years efforts which relied on phone or email responses only (2008/2009 n=778, 2007/2008 n=331, 2006/2007 n=384).

On average responding beekeepers lost 42.2% of their operation, this is an 8 point or 23% increases in the average operational loss experienced by beekeepers in the winter of 2008/2009.

Average losses were nearly 3 times greater than the losses beekeepers reported that they considered acceptable (14.4%). Sixty-one per cent of beekeepers reported losses in excess of what they would consider acceptable.

Colony Collapse Disorder (CCD) is characterized, in part, by the complete absence of bees in dead colonies and apiaries. This survey was not designed to differentiate between definitive cases of CCD and colonies lost as the result of other causes that share the "absence of dead bees" symptom. Only 28% of operations reported that at least some of their dead colonies were found dead without dead bees. However this group lost a total of 44% of their colonies, as compared to the total loss of 25% experienced by beekeepers who did not report losses indicative of CCD.

Responding beekeepers attributed their losses to starvation (32%), weather (29%), weak colonies in the fall (14%), mites (12%), and poor queens (10%). Only 5% of beekeepers attributed CCD as the major cause for their losses.

It is also important to note that this survey only reports on winter losses and does not capture the colony losses that occurs throughout the summer as queens or entire colonies fail and need to be replaced. Preliminary data from other survey efforts suggest that these "summer" losses can also be significant. All told the rate of loss experienced by the industry is unsustainable.

Population Dynamics of honey bee colonies derived from Dutch quantitative monitoring 2006 - 2009

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A longitudinal study of the data produced by beekeepers who responded to Monitor Questionnaires on colony losses in all years (2006-2009) learned that the high losses in the Netherlands were fully compensated by beekeepers who had low losses. Beekeepers with high losses were unable to compensate their losses, also, because they suffered significant higher losses in the years that followed the initially experienced high losses. Based on this observation we try to predict the honeybee mortality risk per beekeeper for the next years. We concluded that the Dutch pollination- and honey production capacity suffered because of replacement efforts by beekeepers with high losses. Next to that we found that the mortality risk was dependent on the province where bees were located. A good indicator for this appeared to be the total number of beekeepers who lost more than 20% of their colonies per province. We propose to use this MR20+ indicator internationally.

Varroa treatment in 2007 and honey bee mortality in the winter 2008-2009

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Dutch COLOSS Questionnaire response data 2008 and 2009 were analyzed to determine possible associations between *Varroa* treatment in 2007 and overwintering mortality 2008-09. The available data set (225 beekeepers with 1710 colonies before winter 2008-2009) showed a substantial variation in *Varroa* treatments. The majority of beekeepers also varied their methods between years.

For our analysis we developed a model based on the timing of treatment with subgroups of varroacides. As a result we found that *Varroa* treatments started in August 2007 and ended before October were associated with a low overwintering mortality 2008-09. Treatments started in September and continued in October or treatments during August, September and October were associated with high overwintering mortality. Within the time groups, application of formic acid had a strong association with low overwintering mortality whereas thymol formulations had a strong association with high overwintering mortality.

Preliminary Results: Honey bee colony losses in Denmark, winter 2009-2010

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The Danish beekeepers' association conducted a survey to estimate winter colony losses for 2009/2010. 679 answers from our 3671 members and 4000 beekeepers, representing 18.5% of the members and 17% of the beekeepers. This survey represents 13657 colonies.

A total loss of 14.2% of managed honey bee colonies was recorded. This is an acceptable loss due to earlier years losses: 7% (07), 32.8% (08), 10% (09). The overall average for the last 15 years is 14.6%. Removing the two biggest losses of 30% (96) and 32.8% (08) the average loss is: 11.7%.

661 beekeepers responded to the on-line survey and an additional 19 answered the essential survey in the beekeeper magazine. In the magazine we stimulated the beekeepers to use the on-line survey. The response rate is slightly higher than last year's survey.

Average losses were close to the beekeepers' expectation of the general losses in the local area. Losses considered acceptable for own operation was 10.41% (n= 537) and 16.1% for the nearby neighborhood.

Only very few colonies were lost due to lack of bees in the colonies in the late fall (how many of your colonies that died between October 1 and April 19, were lost without dead bees either in the hive or in the bee yard?). 35% of the dead colonies had the absence of bees, giving an indication of a kind of Colony Collapse Disorder (CCD) situation.

The beekeepers' personal opinion about the losses is very interesting. They attributed their losses to starvation (17%), weak colonies (25%), varroa (6%), poor queens (26%), nosema (4%) and the "do not know" situation (22%).

It seems likely that starvation, weak colonies and poor queens are the major reasons to the losses, whereas only 6% explained the losses with varroa. This seems to be unlikely, and emphasizes that we in the future years have to have focus on varroa and treatments.

Some of the questions from the 2010 coloss questionnaire need a careful going-over again, even some questions are even a very doubtful character.

Honey bee monitoring and surveys in England and Wales

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Following the publication of the Healthy Bees Plan in April 2009 by the Department for the Environment and Rural Affairs (Defra) additional funding was provided by Defra and the Welsh Assembly Government to carry out a survey to collect baseline information on pest and disease levels in honey bees throughout England and Wales. A statistically based apiary and colony survey is to be carried out over two years, collecting samples from up to 25000 colonies in 5000 apiary sites. These samples are being analysed for a whole range of honey bee pests and diseases using molecular diagnostics. Approximately 2000 samples were collected in the first year and we are now moving into the final year of sample collection. The evidence gathered and a robust analysis of the risks will be used to direct the future bee health plan in England and Wales. In addition to this the NBU is in the second year of conducting an extensive Honey Bee Husbandry Survey on current beekeeping practices in the UK. The information from this survey is being gathered by means of a questionnaire, available both on-line via BeeBase or hard copies circulated to beekeepers through the bee health inspection service at association meetings and training events. This data will be collected annually and be used to monitor trends in UK beekeeping and to assist with beekeeper training. This is the most comprehensive survey of beekeeping practices ever completed in the UK. The Basic CoLoss questionnaire has been incorporated into this survey to allow the information collected in England and Wales through the bee health programme to be fed back into the CoLoss working group. Approx 1,700 responses were received in 2009.

When considering the proportion of changes (losses or gains) in colony numbers between September 2008 and April 2009, there was an overall fall of 13.8%. It is important to note that this colony loss figure was obtained not by averaging losses at the apiary level as presented by individual respondents, but by calculating the overall percentage changes in colony numbers across all colonies and counties. This gives a fairer picture of colony losses, otherwise a beekeeper who had one colony and lost it would be recorded as 100% loss; equally, if he/she obtained just one more hive, this would be a 100% gain; on the other hand, if a beekeeper with 50 colonies loses, this would be recorded as a 2% loss. Given that so many survey respondents have only one or two colonies, if their individual changes (losses or gains) were averaged, this could potentially alter (artificially inflate or deflate) losses by several per cents.



WG4

Standardized protocols for honey bee vitality and diversity



Preliminary evaluations of the Coloss genotype-environment interaction experiment

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As an activity of working group 4, a comparative field experiment to test for the vitality of different genetic origins of European honey bees has been started in July 2009. At 16 different locations all over Europe, 18 different strains and ecotypes of honey bees are being evaluated according to a common protocol.

Preliminary data on colony survival, bee population development, the infestation with Varroa and Nosema and the hygienic behavior will be presented from a part of the test colonies. Methods to optimize the central data collection and statistical analysis will be discussed. The experience of the test participants will be gathered to further standardize the testing method in order to create internationally regarded recommendations for scientists and breeders.

Preliminary data about isoenzyme variability of some *A. mellifera* subspecies involved in the WG4 GEI experiment

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Traditionally, taxonomy of the honey bee *Apis mellifera* has been based on morphology and at present about 26 subspecies are recognized on the basis of classical morphometry. Different biochemical-genetic analyses have been used extensively in studying the genetic diversity of organisms and are appropriate for studying the *Apis mellifera* variability also. Data about isoenzyme polymorphism could be useful for understanding the subspecies discrimination and revealing the existence of hybrid zones between them. Allozymes could also be used as genetic markers in characterization of genetic differentiation among the honey bee races and populations, and in analyzing the phylogeny of *A. mellifera*.

The genetic variability of honey bee populations from four different sampling regions involved in the GEI experiment of COLOSS WG4 has been studied: Pulawy (Poland); Kirchhain (Germany); Halkidiki (Greece) and Plovdiv (Bulgaria). Honey bees from Germany and Poland were recognized as *A. m. carnica*, bees from Greece and Bulgaria – as *A. m. macedonica* and *A. m. rodopica*, respectively. Isoenzymic analysis of six enzymic systems (MDH-1, ME, EST-3, ALP, PGM and HK) corresponding to 6 loci has been done. All loci were found to be polymorphic in most of the populations studied. Three alleles were detected at MDH-1 locus (MDH⁶⁵, MDH⁸⁰ and MDH¹⁰⁰), three alleles at Me locus (ME⁹⁰, ME¹⁰⁰ and ME¹⁰⁶), six alleles - at EST-3 locus (EST⁸⁰, EST⁸⁸, EST⁹⁴, EST¹⁰⁰, EST¹⁰⁵ and EST¹¹⁸), three alleles - at ALP locus (ALP⁸⁰, ALP⁹⁰ and ALP¹⁰⁰), three alleles at PGM locus (PGM⁸⁰, PGM¹⁰⁰ and PGM¹¹⁴) and four alleles at HK locus (HK⁸⁷, HK¹⁰⁰, HK¹¹⁰ and HK¹²⁰). Neighbour-Joining phylogenetic tree and UPGMA dendrogram were obtained by genetic distance matrix methods. Populations studied are grouped in three clades. The populations from Bulgaria and Greece were clustered in the first clade and those from Germany and Poland – in the other two. The research is in progress including other sampling regions from the GEI experiment of COLOSS WG4.

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Morphometric and genetic assignment of foraging bees from a population of *A. m. ligustica* and *A. m. mellifera*

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On the Danish island Læsø both *A. m. ligustica* and *A. m. mellifera* bees have been kept for several years. In 2003 approximately 100 foraging honey bees were collected from 13 different plots in order to get an estimate of the distribution of these two subspecies and hybrids on the island. The sampled bees were assigned as *A. m. ligustica*, *A. m. mellifera* or hybrids based on the colour of their abdominal rings and the cubital index. In addition the mitochondrial haplotype was determined using *DraI* restriction patterns. Based on the colour alone a very high proportion of the bees was assigned as *A. m. mellifera* (82%), few as *A. m. ligustica* (15%) and only a very few as hybrids (3%). The proportion of hybrids increased when both the colour and the CI index were used for the assignment (29%) and even more when the mitochondrial haplotype was included (55%). There was generally a very good correlation between the haplotyping and the CI index.

Contribution to the Coloss genotype-environment interaction experiment in France

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Two different sites are used in France for the Genotype – Environment Interaction Test in the framework of COLOSS. One is located near Toulouse and managed by Olivier Celle, including 30 colonies. The other near Avignon includes 30 colonies managed by Yves Le Conte. The queens were introduced in the colonies late in 2009, just after the Apimondia meeting in Montpellier. Most of the queens were accepted and were ready for wintering.

We will present the update of this experiment, including differences in survival rate, development and behaviour of the different stocks.

Genotype-Environment interaction experiment and evaluation of its part in Turkey

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Evaluation of survival and performance of different races of honey bees under different environments in different geographical areas with representative of marked differences of climatic and phytogeographic characteristics within or across countries will reveal the specific or wide adaptation of honey bee races. Degree of similarity or dissimilarity of experimental locations in terms of ecological conditions is likely to be associated with Genotype-Environment interactions. Having these in consideration and using the richness of honey bee diversity and the presence of very different climatic, floristic, and geographic regions in Turkey, a total of 90 honey bee colonies were set up at three locations, each in a different climate zone for the Genotype-Environment Interaction Experiment in the framework of COLOSS. 10 colonies from each race, *A. m. anatoliaca*, *A. m. caucasica*, and *A. m. carnica* were started at three regions; 1- Ankara (Central Anatolia, semi arid, continental climate), 2- Rize (Black Sea, humid subtropical climate) and 3- Kırklareli (Thrace, Mediterranean climate) in the autumn 2009. In location 1, local race *anatoliaca*, others were *caucasica*, and *carnica*; location 2, local race *caucasica*, others *anatoliaca* and *carnica*; location 3, local race *carnica*, others were *anatoliaca* and *caucasica*.

Initial treatment against Varroa was made with Perizin in November to all colonies.

Update of the experiment after wintering, survival rates, strength of colonies, Varroa infestation and other parameters will be presented and discussed.

Discrimination of *Apis mellifera* subspecies involved in the GEI experiment

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Different approaches to confirm the origin of honey bees are currently in use in Europe. However, there are limited collections of accessible reference data. Using the colonies that are currently part of the common GEI experiment of Working Group 4 within COLOSS, we will use a combination of methods that are generally employed to analyze bee samples for their geographic and genetic origin: morphometric analysis, mtDNA analysis, microsatellites and isozyme analysis. These data will be useful both for the documentation of the genetic origin of each colony involved in the common experiment and for the creation of a published and accessible reference database for honey bee diversity in Europe.

Season and *Varroa destructor* contamination influence on the hygienic behaviour of local Bulgarian honeybee

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The local Bulgarian honey bee named by Petrov (1990) as *Apis mellifera rodopica* is a basis for selective work in Bulgaria. With this regard, a morphometric analysis by specific characteristics has been carried out in order to determine the race belonging of local bees. On the other hand, biochemical-genetic researches of polymorphism in some protein and isoenzyme systems have been carried out for different populations of Bulgarian bees (Ivanova 1996; Ivanova *et al.*, 1998; Ivanova *et al.*, 2007; Ivanova *et al.*, 2008). Hygienic behaviour is an ethological characteristic which has been the object of different studies. The influence of season and degree of *Varroa destructor* contamination on the hygienic behaviour and ascospaerosis larvae sink rate was investigated in this study in order to avoid the mistakes in selection of bee colonies and to create disease-resistant lines of the local Bulgarian honey bee. The observations were conducted during the five-year-period, totally with 99 bee colonies. In order to test the influence on the hygienic behaviour, a rhomboid pattern, the area of which includes 100 capped worker cells, was used. The recording of the cleaned and not cleaned cells is made once every 6 hours, twenty-four-hours a day until their total cleaning. In all tests, a capped worker brood in stages “straight larva” and pupa with “white eyes” was used. The results received in this study show that the hygienic behaviour testing of bee colonies should be conducted during the spring - summer period when the variability of bee colonies does not exceed 6-12 h, while during the autumn period in the same colonies this character varies up to 108 h. It was concluded also that the higher *Varroa destructor* contamination can increase the cleaning instinct of worker bees.

Ivanova E. (1996) Variability of *Apis mellifera* in Bulgaria – ontogenetic and population-genetic aspects. PhD Dissertation University of Plovdiv, Bulgaria, 159pp.

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Colony mortality and morbidity in migratory beekeeping operations in the Eastern United States: A longitudinal descriptive study based on rates of risk factor exposure

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The cause(s) for the increased honey bee colony mortality that has been experienced since 2006 remains unresolved. In this study, we identified and quantified risk factors associated with colony mortality in migratory beekeeping operations in the eastern United States. Overall, mortality among 81 colonies was 58% during the 10-month observation period. The presence of Parasitic Mite Syndrome (PMS) in the brood, a queen 'event', and poor brood patterns each increased the risk of colony mortality by the next inspection (approximately 50 days later). We found no evidence that levels of varroa mites surpassing 5% infestation on adult bees were associated with increased risk of mortality or that colonies would have or develop signs of PMS. To our knowledge, this longitudinal study is the first to systematically assess and quantify the risk for colony death using epidemiologic methods. Our findings of apicultural risks should be verified with studies in other geographic areas.