Action FA0803

COLOSS WORKSHOP

WG 4

"Honey bee vitality and diversity - Field observations of experimental GEI colonies"

26 - 29. 07. 2011

Research Institute of Horticulture
Apiculture Division in Puławy
Kazimierska 2, 24-100 Puławy, Poland

Action FA0803

List of Participants

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Research Institute of Horticulture in Skierniewice

Apiculture Division in Puławy

Region of Puławy

Agricultural School Complex in Pszczela Wola Secondary Technical School of Agriculture

Puławy Town Hall

APIS Apiculture Cooperative in Lublin

Action FA0803

COLOSS Work Shop

"Honey bee vitality and diversity – Field observations of experimental GEI colonies "

26 - 29. 07. 2011

Research Institute of Horticulture

Apiculture Division in Puławy

24-100 Puławy, Kazimierska 2, Poland

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Agenda

TIME	PROVISIONAL PROGRAM
26.07.2011 (Tuesday) Marynka Palace in Puławy	
09:30 - 12:00	WG4 registration, visiting of Marynka Palace with surronding park and Bee Product Quality Testing Laboratory
12:00 – 12:30	Welcome and organizational matters
12:30 – 14:00	Krystyna Pohorecka, Andrzej Bober, Marta Skubida, Dagmara Zdańska, Artur Miszczak, Piotr Sikorski - Occurrence of pathogenic agents in apiaries with increased mortality of bee colonies and properly functioning. Pathogens and residues of pesticide
	Ralph Büchler - Vitality test - experience with a threshold based treatment concept as part of a selection program for increased *Varroa* resistance
	Cecilia Costa, Giacomo Vaccari, Eugenia Oliveri, Marco Lodesani - Survival of colonies in the Italian GEI test locations
	Discussions

14:00 – 15:00	Lunch
15:00 – 16:00	Beata Bąk, Maciej Siuda, Jerzy Wilde - Various methods of *Varroa destructor* control
	Lauri Routtinen, Seppo Korpela – The effect of hard winter circumstances to food consumption and winter survival of different bee strains in Finland
	Małgorzata Bieńkowska, Jerzy Wilde, Beata Panasiuk, Paweł Węgrzynowicz, Dariusz Gerula – Performance of bee colonies of the GEI experiment in Poland
	Discussions
16:00 – 16:30	Coffee break
16:30 – 19:30	Visit to the Old Town - Kazimierz Dolny
20:00	Welcome dinner
27.07.2011 (Wednesday) Seasonal Laboratory of Apiculture Division	
09:00 – 10:30	Andrzej Oleksa - Conservation genetics of dark honey *Apis mellifera mellifera* in Poland
	Evgeniya N. Ivanova, Malgorzata Bienkowska, Plamen Petrov, Beata Panasiuk, Ivan Stoyanov - Allozyme polymorphism in *Apis mellifera* subspecies selectively reared in Poland and Bulgaria
	Evgeniya Ivanova, Maria Bouga, Teodora Staykova, Sladjan Rasic, Leonidas Charistos, Mica Mladenovic, Plamen Petrov, Ivan Stoyanov, Fani Hatjina - Study on Balkan honey bees' genetic variability based on alloenzymic analysis
	Marina Meixner, Maria Bouga, Leonidas Charistos, Per Kryger, Evgeniya Ivanova, Fani Hatjina - Genetic variability of honey bee origins used in the GEI experiment using geometric morphometrics approach
	Discussions
10:30 – 11:00	Coffee break
11:00 – 13:30	Maja Drazic, Janja Filipi, Ivan Pavlović, Ivica Grgurić, Ivan Mihaljević, Nikola Kezić - Apiaries and Incidence of Nosema at Island of Unije
	Anna Gajda, Urszula Grzęda, Grażyna Topolska - The course of *Nosema* infection in experimental GEI colonies
	Dariusz Gerula, Paweł Węgrzynowicz, Beata Panasiuk, Małgorzata Bieńkowska – *Varroa destructor* infestation on ABPV and DWV incidence and wintering of colonies
	Teresa Szczęsna, Krystyna Pohorecka, Ewa Waś, Helena Rybak-Chmielewska, Monika Pytlak, Katarzyna Kachaniuk - Acaricide residues in beeswax from apiaries with increased mortality of bee colonies and properly functioning

	Discussions
13:30 – 14:30	Lunch
14:30 – 15:30	Discussion on GEI experiment
15:30 – 16:30	Visit to the GEI experimental apiary in Bronowice
16:30	Trip to Wierzchoniów- excursion to loess ravines
20:00 – open	Social dinner
28.07.2011 (Thursday) Seasonal Laboratory of Apiculture Division	
08:30 – 11:00	Discussion on GEI experiment
11:00 – 11:30	Coffee break
11:30 – 12:15	Discussion on possible joint publication and other matters
12:15 - 14:30	Trip to Lublin- visit to APIS Apiculture Cooperative, the leading producer of meads and natural honey products in Poland
14:30 – 17:30	Lunch in Pszczela Wola Visit to apiary of Agricultural School Complex Vocational Training Centre in Pszczela Wola (lunch) Future developments and issues for discussion at joint workshop and Coloss Conference in Serbia
18:00 – 19:30	Visit to old forge in Wojciechów (with demonstrations)
20:00	Dinner in Puławy
29.07.2011 (Friday) Marynka Palace in Puławy	
10:00 – 11:30	Discussion on future plan activities of WG4. Concluding of the meeting
11:30 – 12:00	Coffee break
12:00 – 16:00	End of the workshop and possible visit in Breeding Apiary in Końskowola, visit in Janowiec (for those who leave on 30th of July)

Deadline for registration and abstracts: 15 June 2011

Local Organizers: Małgorzata Bieńkowska, Beata Panasiuk

Abstracts

1. Beata Bąk, Maciej Siuda, Jerzy Wilde - Various methods of *Varroa destructor* control
2. Małgorzata Bieńkowsk, Jerzy Wilde, Beata Panasiuk, Paweł Węgrzynowicz, Dariusz Gerula - Performance of bee colonies of the GEI experiment in Poland
3. Ralph Büchler - Vitality test - experience with a threshold based treatment concept as part of a selection program for increased *Varroa* resistance
4. Cecilia Costa, Giacomo Vaccari, Eugenia Oliveri, Marco Lodesani - Survival of colonies in the Italian GEI test locations
5. Maja Drazic, Janja Filipi, Ivan Pavlović, Ivica Grgurić, Ivan Mihaljević, Nikola Kezić - Apiaries and Incidence of Nosema at Island of Unije
6. Anna Gajda, Urszula Grzęda, Grażyna Topolska - The course of *Nosema* infection in experimental GEI colonies
7. Dariusz Gerula, Paweł Węgrzynowicz, Beata Panasiuk, Małgorzata Bieńkowska - *Varroa destructor* infestation on ABPV and DWV incidence and wintering of colonies
8. Evgeniya N. Ivanova, Malgorzata Bienkowska, Plamen Petrov, Beata Panasiuk, Ivan Stoyanov - Allozyme polymorphism in *Apis mellifera* subspecies selectively reared in Poland and Bulgaria
9. Evgeniya Ivanova, Maria Bouga, Teodora Staykova, Sladjan Rasic, Leonidas Charistos, Mica Mladenovic, Plamen Petrov, Ivan Stoyanov, Fani Hatjina - Study on Balkan honey bees' genetic variability based on alloenzymic analysis
10. Marina Meixner, Maria Bouga, Leonidas Charistos, Per Kryger, Evgeniya Ivanova, Fani Hatjina - Genetic variability of honey bee origins used in the GEI experiment using geometric morphometrics approach
11. Andrzej Oleksa - Conservation genetics of dark honey *Apis mellifera mellifera* in Poland
12. Krystyna Pohorecka, Andrzej Bober, Marta Skubida, Dagmara Zdańska, Artur Miszczak, Piotr Sikorski - Occurrence of pathogenic

agents in apiaries with increased mortality of bee colonies and properly functioning. Pathogens and residues of pesticide

13. Lauri Routtinen, Seppo Korpela – The effect of hard winter circumstances to food consumption and winter survival of different bee strains in Finland
14. Teresa Szczęsna, Krystyna Pohorecka, Ewa Waś, Helena Rybak-Chmielewska, Monika Pytlak, Katarzyna Kachaniuk - Acaricide residues in beeswax from apiaries with increased mortality of bee colonies and properly functioning

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Various methods of *Varroa destructor* control

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In the assay various patterns of controlling *Varroa destructor* were being applied. The aim of the study was to assess which of the methods is most efficient. In the autumn 2009, a hundred bee colonies not treated for *Varroa* were selected, out of which three experimental groups were randomly created (25 colonies in each group) in the spring 2010, based on the applied method for treating *varroosis*: group I (CH) – summer treatment mostly, with the use of chemotherapy; group II (IT) – integrated treatment, i.e. treating the mite with various methods several times a year; group III (N) – only natural ways of controlling the mite with the use of essential oils and organic acids; and group IV (C) – control group, not treated for *varroosis*.

In each group the methods for treating bee colonies for *Varroa* mite were selected based on the availability of substances registered in Poland, such us: Bayvarol (active substance - flumetrine), Apiwarol and Biowar (active substance - amitraz) and Api Life Var (main components - ethereal oils). In group II (IT), additionally, drone brood was being removed. In the spring 2010, before any of the patterns were applied, the bees had been infected with *Varroa destructor* mites, with the highest intensity of infection in group C (3.92%), and lowest in group IT (0.14%). In the fall, after the treatment , the highest degree of parasite infection was observed in the samples from group C, amounting to 3.25%, while in the samples from the groups treated for *varroosis* the degree of infection ranged from 0.14% in group N to 0.35% in group CH. The average colony strength (the number of frames

covered with bees during the season) in individual groups did not differ statistically and ranged between 7.3 in group CH and 7.9 in group C. In 2010, in the dead bees coming from all groups *N. apis* and *N. ceranae* spores were found. Forty percent of all tested bee colonies were affected by these sporidia, yet in only 3% of the colonies the degree of infection was high. No relationship between the degree of bee colonies infection with *Nosema sp*. and the applied *varroosis* treatment was observed. The highest costs of controlling *Varroa destructor* in 2010 were generated in group N (312.85 €), and lowest - in group CH (130.89 €).

In the spring 2011, before any of the patterns were applied, the bees had been infected with *Varroa destructor* mites, with the highest intensity of infection in group C (4.07%), and lowest in group N (2.18%). In April 2011 the treatment of groups IT and N with Api Life Var was carried out. The effectiveness of this substance in both groups proved to be lower than 55%.

In all groups *N. apis* and *N. ceranae* spores were found in approximately half of the samples. In 12.5% of the colonies in group I and in 14.28% of the colonies in group II the degree of infection with these sporidia was high. Only in the colonies of group III a high degree of infection was not observed.

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Performance of bee colonies of the GEI experiment in Poland

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Three experimental apiaries were established in Poland in 2009 for evaluation of GEI bee colonies. They were set in different areas of Poland. Altogether 126 bee colonies with queens belonging to 8 populations were placed in the apiaries: 37 colonies in Kunki, 44 colonies in Puławy and 45 in Olsztyn.

Each year of evaluation and in each apiary, similar and the lowest strength of colonies regardless the genotype was in spring time. Honey harvest was different for each location. Hygienic behavior of bees is affected by the genotype. Significant effect of location of the apiary was stated in *Varroa* infestation level. It is surprising that in the apiary that brood removal was not applied, mite infestation was the lowest.

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Vitality test - experience with a threshold based treatment concept as part of a selection program for increased *Varroa* resistance

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Usually, colonies are uniformly treated with acaricides against Varroa soon after the final honey harvest, without regarding their individual infestation level. Due to the generalized treatment, susceptible colonies thus get a chance to rear healthy winter bees, while the relative resistance of other colonies can easily be overlooked.

In order to emphasize any differences in Varroa infestation, we started to establish a threshold-based treatment concept that we call “vitality test” in 2004. At the end of July, after finishing the routine performance test, we select productive and gentle colonies with less than 2% Varroa infestation of their adult bees. These colonies are observed further, but not treated. Until October, the bee population and the Varroa infestation of bees are checked in 3 week intervals. Our aim is to identify untreated breeder colonies with a sustainably low rate of Varroa reproduction and good overwintering abilities.

So far, data from about 470 colonies tested at the bee institute in Kirchhain and by private German bee keepers have been evaluated to define relevant threshold values and to describe the influence of environmental and genetic effects on the survivability without chemical treatments. Some practical recommendations to establish the vitality test as a routine in selection programs on Varroa resistance will be concluded.

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Survival of colonies in the Italian GEI test locations

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The Italian apiaries of the GEI experiment are located in the Po Plain (Northern-Central Italy) and in Sicily. In each apiary 3 geographic origins were present: *Apis mellifera ligustica* from Italy, *A. m. ligustica* from Finland and *A. m. siciliana*.

Colonies were set up uniformly during the summer of 2009. Due to logistic reasons queens of Finnish origin were introduced in the Sicilian apiary in September, and their build up was handicapped. Data collection and colony management was carried out according to the common protocol agreed among Working Group 4 members.

In the Sicilian apiary 45% of the Italian *A. m. ligustica* were lost by spring 2010 and 100% by December 2010. Of the *A. m. siciliana* colonies, 15% were lost by spring 2010 and 35% by December 2010.

In the Po Plain apiary 68% of the *A. m. siciliana* colonies were lost by spring 2010 and 96% by October 2010. Of the Italian *A. m. ligustica* colonies, 54% were lost by spring 2010 and 71% by October 2010. High levels of Nosema spores were detected in all colonies.

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Apiaries and Incidence of Nosema at Island of Unije

Maja Drazic[1], Janja Filipi[2], Ivan Pavlović[3], Ivica Grgurić[3], Ivan Mihaljević[3] and Nikola Kezić[3]

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Experimental apiary with 70 colonies originating from seven European lines was established in summer 2009 at isolated Island of Unije. The lines were equally distributed on five distant locations at the Island. Nosema infestation was examined in autumn 2010 and spring 2011 on survived colonies. The highest average number of Nosema spores in 2010 was at apiary “Vele Stijene” and in 2011 at apiary “Maracuol”.

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The course of *Nosema* infection in experimental GEI colonies

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At the Laboratory of Bee Diseases of Faculty of Veterinary Medicine at Warsaw University of Life Sciences we examined live and dead bee samples from GEI colonies (maintained in Pulawy and Olsztyn) for the presence of *Nosema apis* and *Nosema ceranae*. Live bees were collected usually at the entrances of the hives in the summer. Dead bees were collected at the bottom boards of the hives during the winter. Examination of live forager bees was performed using PCR (to establish *Nosema* species) and also light microscopy (spore counts). Examination of dead bees was done using light microscopy (spore counts).

The results of the investigation of dead bees collected from the hive bottom boards at the end of two winters (2009/2010 and 2010/2011) suggest, that in 82% of the colonies the level of infection increased, while in 18% it decreased, and in 7% it did not change. The increase was the highest in Croatian line (in Pulawy and in Olsztyn) and also in both Kortowka lines. The decrease was most evident in Bulgarian line (in 50% of colonies).The spore count analysis in case of live bee samples is complicated because of the difference between sampling methods (at the entrance of the hive and from the outer frame) and will be discussed.

In 2009 colonies free from *Nosema* infection belonged to Austrian line in Olsztyn, Bulgarian and Kortowka lines in Pulawy. In 2010 in 50% of these colonies *N. ceranae* or *N. apis* + *N. ceranae* appeared (in all colonies from Bulgarian line and in 66% of colonies from Austrian line). In 2009 the mixed infection by *Nosema apis* and *Nosema ceranae* was more common than infection by one species of *Nosema* and was detected

in 63% of colonies. Only in the case of Kortowka the mixed infection was found in 33% of colonies. In 2010 in all the lines mixed infection was found in less than 35% of the colonies. In 33% of colonies in which in 2009 *N. apis* or *N. apis + N. ceranae* were detected, in 2010 only *N. ceranae* was found.

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Varroa destructor infestation on ABPV and DWV incidence and wintering of colonies

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Research Institute of Horticulture, Apiculture Division, Puławy, Kazimierska 2, 24-100 Puławy

The research was conducted in 2010. Two groups of bee colonies were tested:

- colonies with queens inseminated with mixed semen collected from drones belonging to few lines of carniolan bees
- colonies with queens inseminated with semen collected from drones from a single colony

The number of *Varroa* mites was checked at the late summer treatment. Also the number of bees and mites fallen on the bee hive bottom was monitored during winter as well as ABPV and DWV incidence in dead bees collected in December 2010 was checked.

Similar number of both, dead bees and Varroa mites was found in both groups of bee colonies. Also a similar percentage of colonies was infested with viruses in experimental groups.

Colonies that were infected with viruses were observed to be weaker in the spring 2011 comparing to autumn 2010. The strength of colonies that were infected with ABPV was reduced in spring for 8.4% (average percentage of frames removed from colonies in spring) while the strength of colonies free of this virus was reduced for 7.7%. Instead, the strength of colonies infected with DWV was reduced for 22.8% and free of the virus for only 7.2%.

It was stated that colonies were infected with only one virus: ABPV or DWV. None of the colonies was lost during winter season. However five queens were lost so the colonies were removed from the experiment in spring time.

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Allozyme polymorphism in *Apis mellifera* subspecies selectively reared in Poland and Bulgaria

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The genetic variability of honey bee populations of three subspecies selectively reared in Poland (*A. m. carnica* and *A. m. caucasica*) and Bulgaria (*A. m. macedonica* – type *rodopica*) has been studied using isoenzymic analysis of six enzymic systems (MDH-1, ME, EST-3, ALP, PGM and HK) corresponding to 6 loci. All loci, were found to be polymorphic in of the populations studied. Three alleles were detected at MHD-1 (MDH^{65}, MDH^{80} and MDH^{100}), Me (ME^{90}, ME^{100} and ME^{106}), EST-3 (EST^{94}, EST^{100} and EST^{118}), ALP (ALP^{80}, ALP^{90} and ALP^{100}), PGM (PGM80, PGM^{100} and PGM^{114}) and HK (HK^{87}, HK^{100} and HK^{110}) loci. The observed and expected heterozygosities (H_o and H_e) ranged from 0.196 (*A. m. macedonica* SM) to 0.265 (*A. m. carnica* MV) and from 0.224 (*A. m. macedonica* SM) to 0.273 (*A. m. carnica* GR), respectively. Allele frequencies of all loci were used to estimate Nei's (1972) genetic distance, which was found to range from 0.003 (between *A. m. macedonica* TR and SM and between *A. m. carnica* GR and MV populations) to 0.057 (between *A. m. macedonica* SM and *A. m. caucasica* populations). The estimated mean F_{ST} value from allozyme data was 0.0364. UPGMA dendrogram was obtained by genetic distance matrix methods; *A. m. macedonica* (type *rodopica*), *A. m. carnica* and *A. m. caucasica* populations studied are grouped in different clades.

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Study on Balkan honey bees' genetic variability based on alloenzymic analysis

Evgeniya Ivanova[1*], Maria Bouga[2], Teodora Staykova[1], Sladjan Rasic[3], Leonidas Charistos[4], Mica Mladenovic[3], Plamen Petrov[5], Ivan Stoyanov[1], Fani Hatjina[4]

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The genetic variability of honey bee populations from eleven different regions of Bulgaria, Greece, Serbia and Montenegro has been studied using alloenzymic analysis of six enzymic systems (MDH-1, ME, EST-3, ALP, PGM and HK) corresponding to 6 loci. All loci were found to be polymorphic in most of the populations studied. Four alleles were detected at Mdh-1 locus (MDH^{65}, MDH^{80}, MDH^{100} and MDH^{125}), three alleles at ME locus (ME^{90}, ME^{100} and ME^{106}), six alleles - at EST-3 locus (EST^{80}, EST^{88}, EST^{94}, EST^{100}, EST^{105} and EST^{118}), three alleles - at ALP locus (ALP^{80}, ALP^{90} and ALP^{100}), two alleles at PGM locus (PGM^{100} and PGM^{114}) and four alleles at HK locus (HK^{87}, HK^{100}, HK^{110} and HK^{120}). There was found, that ME^{100} allele was fixed in the Serbian populations and EST^{100} allele - in one of the Greek populations studied. The observed and expected heterozygosities (H_o and H_e) ranged from 0.161 to 0.276 and 0.222 to 0.335, respectively. Allele frequencies of all loci were used to estimate Nei's (1972) genetic distance, which was found to range between 0.001 (between one Serbian and

one Montenegro population) and 0.101 (between one Serbian and one Greek population). The estimated mean F_{ST} value from allozyme data was 0.094.

Neighbor-Joining phylogenetic tree and UPGMA dendrogram were obtained by genetic distance matrix methods. Populations studied are grouped in two clades: The populations from Bulgaria and Greece were clustered in the first clade and these from Serbia and Montenegro – in the second one.

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Genetic variability of honey bee origins used in the GEI experiment using geometric morphometrics approach

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One of the main goals of COLOSS WG 4 is to establish a common protocol for the discrimination of honey bee populations. In Europe, several different methods are used to determine the subspecific origin of honey bees. In WG4 different methods are currently applied to analyze samples of the colonies that are part of the common GEI experiment.

The geometric morphometrics analysis is based on coordinates of 19 landmarks located at vein intersections of the left wing. The data are statistically processed using MORPHOJ and NTSYS software packages.

The results of the geometric morphometric analysis will be combined with the results from microsatellites, mtDNA analysis, isoenzymic and classical morphometric analysis. The results will contribute to the documentation of the genetic origin of each colony and to the establishment of a published and accessible reference database that will be of value to scientists and apiculturists working in the field of European honey bee biodiversity and conservation.

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Conservation genetics of dark honey *Apis mellifera mellifera* in Poland

Andrzej Oleksa

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Genetic variability of the European western and northern subspecies of the honey bee, *Apis mellifera mellifera*, is currently severely endangered by hybridization with introduced bees of evolutionary lineage C. In recent decades non-native bees (predominately Carniolan bees, *Apis m.carnica*) were introduced into bee breeding, mainly from south-eastern Europe, but it was expected that in Poland genetic mixing would be somewhat delayed as compared to that in Western Europe. In the 1970's, a conservation breeding program was established in order to preserve the genetic diversity of the remaining native honeybees in northeast Poland, believed to be one of the last haunts of Polish native dark bees. Here I present investigations into those populations using mitochondrial and nuclear DNA markers.

Action FA0803

Occurrence of pathogenic agents in apiaries with increased mortality of bee colonies and properly functioning. Pathogens and residues of pesticide*

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The aim of investigations was the identification of pathogens and residues of pesticides used in agriculture in samples (dead bees, stores of food and brood nest wax) sent from apiaries where mass losses of bee colonies were observed. Material for laboratory tests was collected between autumn 2009 and spring 2010, from apiaries where: losses of bee colonies were above 10% and colonies with CCD symptoms were observed , losses of bee colonies were above 10% but no colonies with CCD symptoms were observed and losses of bee colonies were up to 10 %. In each apiary samples were collected separately from several colonies. Laboratory examinations were carried out to detect the presence of *V. Destructor* (shaking method), *Nosema* spp. (microscopically using haemocytometer), chronic bee paralysis virus (CBPV), acute bee paralysis virus (ABPV), deformed wing virus (DWV) and Israeli acute paralysis virus (IAPV) (RT-PCR method) and pesticide residues (GC/MS method). In 2010 we have collected material for the laboratory studies from 295 apiaries. In 231 of them the mass losses above 10% of bee colonies (54% dead of bee colonies on

average) were observed. In this group of apiaries, Colony Collapse Disorder (CCD) symptoms were observed in 112 of them (48,5%). Statistical analysis of tests results showed that in apiaries with mass bees mortality, the presence of *Varroa* mites, ABPV and DWV viruses were detected most frequently compared to the apiaries with low losses. There was also higher the level of *Varroa* infestation. The presence of 3 or 4 pathogens were simultaneously detected in 70% of honeybee colonies. In apiaries where losses of bee colonies were up to 10 % the presence of 3 or 4 pathogens were simultaneously detected in 24% of honeybee colonies only. The residues of pesticides was detected in only a few samples of dead bees and beebread.

Action FA0803

The effect of hard winter circumstances to food consumption and winter survival of different bee strains in Finland

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The facts behind successful overwintering of honeybees have been discussed widely during the time that bees have been kept in areas with cold winters. Both beekeepers and researchers have estimated the roles of population strength, food consumption, genetic fitness and management techniques in successful overwintering.

It is necessary to know the key factors of a dead beehive. This makes it possible to analyze the reasons for losses and produce comparable and reliable data. Attaching this to the data received from bee samples tested for varroa levels, nosema, virus and AFB would make it possible to produce a reliable picture of the cause that led to winter loss. By introducing the use of a *loss record card* immediately after the colony has died, it would be possible to minimize subjective errors (compare COLOSS wg1).

After the analysis the reasons for winter losses could be divided in three different categories. Management failures, outer disturbances and lack of genetic fitness. Also the climate condition seems to have a regulative effect for the overwintering of honeybees. All of these factors can be influenced by beekeepers.

The minimum strength of a bee colony seems to be 500 % of frame surfaces covered by bees, food stores and consumption by themselves seem not to be deciding factors. Also the movement capability of the winter cluster is important. The longevity of bees depends on the beginning time of the brood rearing in the spring and the pollen stores both in fall and spring. More data on overwintering is required to improve the overall survival rate.

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Acaricide residues in beeswax from apiaries with increased mortality of bee colonies and properly functioning*

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In 2010, under monitoring studies of the most commonly acaricides used for *Varroa* control in Poland, serial measurements of these substances in beeswax harvested from comb foundation (13 samples) and brood nest wax (196 samples) were performed. The samples were collected from the apiaries in which there was massive loss of bee colonies (above 10%) and from apiaries in which this phenomenon was not observed (loss of less than 10%). The study included the following substances: 2.4 – dimethylphenylformamide (DMF), tau-fluvalinate, flumethrin, bromopropylate, acrinathrin, coumaphos, deltamethrin. Since DMF is the major degradation product of amitraz, this substance was selected for amitraz residues monitoring in beeswax.
SPE technique was used for acaricides extraction from beeswax using Clearnet Florisil–SPE 1000mg/6ml Column (Agela Technologies). DMF determinations were performed using GC/MS technique on Gas Chromatograph Mass Spectrometer (GCMS-QP 2010 Plus, Shimadzu) and ZB-5HT INFERNO 20m x 0.18mm x 0.18µm chromatographic

column (Phenomenex). The identification of the DMF was carried out with full SCAN mode, and the quantification with SIM (selected ion monitoring). Other above-mentioned acaricides were determined by gas chromatography with electron capture detector (GC/ECD) on DB-35MS 30m x 0.25mm x 0.25µm Column (Agilent J&W GC Column).

All samples of comb foundation were free from residues of DMF, flumethrin and acrinathrin, one sample contained bromopropylate, three samples – coumaphos and as many as 12 samples - fluvalinate. Among 196 samples of beeswax, 98 samples (50%) were free from all tested acaricides, the remaining 98 contained at least one active substance. The largest contamination was noted for fluvalinate and coumaphos. Fluvalinate were detected in 66 samples, representing about 34% of all samples, coumaphos residues – in 30 samples (15%). Among other tested acaricides, DMF was detected in 13 samples (6.6%), bromopropylate – in 8 samples (4.1%), acrinathrin – in 4 samples (2.0%) and deltamethrin – in one sample (0.5%).