Action FA0803

COLOSS WORK SHOP

WG4

BEE BOOK
AND DATA ANALYSES OF ECOTYPE – ENVIRONMENT INTERACTIONS EXPERIMENT

23[th] – 27[th] January 2012

MTT Agrifood Research Finland,
Jokioinen, Finland
ww.mtt.fi
HAMK University of Applied Sciences
Mustiala, Finland
www.hamk.fi

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Local organizer

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Local Organizing committee:

Contact to:

Lauri Ruottinen	Email: lauri.ruottinen@mtt.fi	+358 40 547 3652
Merja Vainio	Email: merja.vainio@hamk.fi	+358 364 65 542
Seppo Korpela	Email: seppo.korpela@mtt.fi	+358 44 084 4582
Sakari Raiskio	Email: sakari.raiskio@mtt.fi	+358 40 283 3002
Lassi Kauko	Email: lkauko@netti.fi	+358 50 545 3628

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CONTENTS:

List of participants

Bienkowska	**Malgorzata**	malgorzata.bienkowska@man.pulawy.pl
Bouga	**Maria**	mbouga@aua.gr
Büchler	**Ralph**	ralph.buechler@llh.hessen.de
Çakmak	**Ibrahim**	icakmak@uludag.edu.tr
Costa	**Cecilia**	cecilia.costa@entecra.it
Francis	**Roy**	royfrancis.mathew@agrsci.dk
Fuchs	**Stefan**	s.fuchs@bio.uni-frankfurt.de
Gryger	**Per**	per.kryger@agrsci.dk
Hatjina	**Fani**	fhatjina@instmelissocomias.gr
Invanova	**Evgeniya**	geneiv@uni-plovdiv.bg
Kantanen	**Juha**	juha.kanatanen@mtt.fi
Kezić	**Nicola**	nkezic@agr.hr
Korpela	**Seppo**	seppo.korpela@mtt.fi
Kauko	**Lassi**	lkauko@netti.fi
Le Conte	**Yves**	leconte@avignon.inra.fr
Drazic	**Maja**	mdrazic@hpa.hr
Mateja	**Janeš**	mateja_janes@yahoo.com
Meixner	**Marina**	marina.meixner@llh.hessen.de
Panasiuk	**Beata**	Beata.Panasiuk@man.pulawy.pl
Raiskio	**Sakari**	sakari.raiskio@mtt.fi
Ruottinen	**Lauri**	lauri.ruottinen@mtt.fi
Uzunov	**Aleksandar**	uzunov@zf.ukim.edu.mk
Wilde	**Jerzy**	Jerzy.wilde@uwm.edu.pl

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COLOSS Workshop
Bee Book and data analyses of GEI experiment
23th January. – 27th January, 2012
MTT Agrifood Research Finland, 31600 Jokioinen, Finland

Agenda 1.st version

TIME	PROGRAM
23.01. 2012 Monday	
	Arrival to Helsinki, transportation / bus to Forssa (130 km)
18:00 ->	Accomodation (in HAMK), Anttila and Heikkilä houses and gathering to dinner (time to dine to 21:30 at Iso Piippu)
24.01.2012 Tuesday	**HAMK, university of Applied Sciences, auditorium and group work rooms**
08:00 - 9:00	Breakfast and Registration at **Huttula**
09:15 - 12:00	Welcome and organizational matters, work shop program (items: publications, Bee Book and future collaboration), working groups and practical arrangements **GEI experiment publication session** starts: Ralph Büchler, Beata Panasiuk, Malgorzata Bienkowska, Seppo Korpela, Sreten Andonov **Effects of genotype and environmental factors on the survivability of European honey bee colonies** Aleksandar Uzunov, Malgorzata Bienkowska, Ralph Buchler, Leonidas Charistos, Fani Hatjina, Nikola Kezic, Beata Panasiuk **Analysis of honey bee ethology respond as a consequence of genotype-environment interaction in the international experiment of COLOSS WG 4** Per Kryger, Marina Meixner, Maria Bouga, Roy Francis **Results from the microsatellite analysis of honey bees used in GEI Experiment** Yves Le Conte **Contribution to bee Book and GEI experiment** Fani Hatjina, Leonidas Charistos **Survival of honey bee colonies of the GEI experiment in Greece as a result of Varroa prevalence**
12:00 – 13:00	Lunch **(Huttula)**
13:00 - 14:30	**GEI experiment publication session** continues
14:30-15	Coffee brake **(Huttula)**
15 - 17:30	Working groups on manuscripts
18:00 – 21.30	Demonstrating over wintering bee hives and discussion about the overwintering physiology at two log open fire with dining in the forest

25.01.2012 Wednesday	HAMK, university of Applied Sciences, auditorium and group work rooms
08:00 - 8:45	Breakfast **(Huttula)**
9:00 – 12.00	Working group summaries from the **GEI experiment publishing groups** and "what has to be done" discussion, closing of the publication working group session
12:00 - 13:00	Lunch (Huttula)
13:00 - 14:30	**Bee Book session**: Introduction to the items: Genetic diversity; Marina Meixner & al. Queen rearing section and genetic and selection; Ralph Büchler & al.; presentations if needed: Nikola Kezić, Maja Drazic, Janja Filipi , Stefan Berg **Colony management on testing apiary**
14:30 - 15:00	Coffee brake **(Huttula)**
15:00 – 17:00	Working groups for the Bee Book materials, short summaries of Bee book working groups and "what to do" discussion, closing the session
18:00 – 22.30	Middle age evening in the University of Applied Sciences brewery Iso Piippu
26.01.2012 Thursday	**MTT Agrifood Research Finland is Finland**
08:00 – 9:10	Breakfast **(Huttula),** moving to MTT, Jokioinen (20 km/ 20 minutes)
9:15 - 12:00	**Future collaboration session** Stefan Fuchs, Ibrahim Cakmak **Stabilizing the Varroosis parasite host system in beekeeping practice. 1. A concept to mimic natural live or die selection without colony losses** Evgeniya Neshova Ivanova, Stanimila Romanova Nikolova **DNA bank establishment from *Apis mellifera* populations inhabiting the territory of Bulgaria** Cecilia Costa **Organization of breeding activity in Italy and coordinated genetic evaluation: limits and perspectives** Maja Drazic, Mateja Janeš, Janja Filipi, Dragan Bubalo, Nikola Kezić **Carnica ecotypes in different environments** Lauri Ruottinen, Juha Kantanen **Conservation of the Nordic Bee (*Apis mellifera mellifera*)**
12:00 - 13:00	Lunch
13:30 - 16:00	Preparing the future project plans in working groups. Coffee and Summaries of the new project plan, discussion and decision action plan.
17:30 -	Dinner at Iso Piippu, Sauna and swimming in the hole in the ice or snow drift (for those who dare to do that)
27.01.2012 Friday	
09:00 - 17:00	Departure. Early breakfast for the early birds in the occupation. Others breakfast 8:00-9:00, Transportations to the airport or airport bus.

Local Organizing committee:

Lauri Ruottinen	Email: lauri.ruottinen@mtt.fi	Tel.: +358 40 547 3652
Merja Vainio	Email: merja.vainio@hamk.fi	Tel:, +358 3 646 5542
Seppo Korpela	Email: seppo.korpela@mtt.fi	Tel.: +358 44 084 4582
Sakari Raiskio	Email: sakari.raiskio@mtt.fi	Tel.: +358 40 283 3002
Lassi Kauko	Email: lkauko@netti.fi	Tel.: +358 50 545 3628

Effects of genotype and environmental factors on the survivability of European honey bee colonies

Ralph Büchler[1]*, Beata Panasiuk[2], Malgorzata Bienkowska[2], Seppo Korpela[3], Sreten Andonov[4)]

[1] LLH, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany
[2] Institute of Horticulture, Apiculture Division, 24-100 Pulawy, Poland
[3] MTT, Agrifood research Finland, 31600 Jokioinen, Finland
[4] Faculty for Agricultural Science and Food, bul. Aleksandar Makedonski b.b., 1000 Skopje, Republic of Macedonia

*ralph.buechler@llh.hessen.de

In order to better understand the role of bee genetics for the Europe-wide occurrence of colony losses an experiment on genotype – environment interactions (GEI) was started by Coloss working group 4 in July 2009.

621 honey bee colonies, representing 18 different genotypes, are comparatively tested in 16 apiaries across Europe. The colonies are kept without any chemical treatments against *Varroa destructor* and other diseases. Colony and queen survival are registered continuously, besides bee population development, productivity, feed balance, swarming, gentleness, hygienic behavior and the infestation with Varroa, Nosema and viruses.

A preliminary evaluation shows cumulative colony losses of 69,5 % until the end of summer 2011 with most of the losses occurring during the winter periods 2009/2010 (18,2 %) and 2010/2011 (31,6 %) resp. Besides problems with the queens (23,1%), most losses were linked with symptoms of Varroa disease (25,6%), Nosema or defecation (7,9%) or weakness and robbery (5,8%). Large differences are observed between the genotypes and the test locations, and significant interactions between genotype and environment indicate the relevance of local adaption for the survivability of colonies.

During the workshop in Finland the latest status of the ongoing experiment will be analysed and the results will be discussed with regard to a final publication in JAR and recommendations to beekeepers and public authorities.

Analysis of honey bee ethology respond as a consequence of genotype-environment interaction in the international experiment of COLOSS WG 4

Aleksandar Uzunov[1]*, Malgorzata Bienkowska[2], Ralph Buchler[3], Leonidas Charistos[4], Fani Hatjina[4)], Nikola Kezic[5)], Beata Panasiuk[2)]

[1] Faculty for Agricultural Science and Food, bul. Aleksandar Makedonski b.b., 1000 Skopje, Republic of Macedonia
[2]Institute of Horticulture, Apiculture Division, 24-100 Pulawy, Poland
[3]LLH, Bee Institute, Erlenstrasse 9, 35274 Kirchhain, Germany
[4]Hellenic Institute of Apiculture (H.A.O. "Demeter"), N. Moudania, Greece
[5]Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia

*uzunov@zf.ukim.edu.mk

In order to evaluate genotype-environment interactions of honey bee vitality and colony losses, international experiment with 621 honey bee colonies from 18 genotypes in 16 test apiaries across Europe was launched in 2009. No chemical treatments against *Varroa destructor* and other diseases were applied since 2011.

Beside testing and evaluation of colony development, productive traits and diseases incidences in the experimental colonies, we additionally have evaluated swarming, defensive and hygienic behaviour. During regular seasonal censuses, a four point graduation scoring system was used for the evaluation of the first two behaviour traits. Additionally, for estimation of hygienic behaviour, field test (pin-test) was used at least two times per active season.

In the first complete season (2010) 419 colonies, 67.5% from the total number of 621, were assessed for defensive and swarming behaviour. During the regular seasonal censuses in the following year, 136 colonies or 21.9% were assessed for both mentioned traits. Regarding the hygienic behaviour, 314 (50.6%) colonies out of starting 621 colonies, were tested and evaluated in 2010. However, due to the low survival rate in the following 2011, pin test was applied only on 121 colonies (19.5%).

The preliminary results from the statistical analysis of the observed ethological traits show strong genotype, environmental and seasonal influence. Additional rate differences for hygienic behaviour appeared as a consequence of application of various time intervals (8, 12 or 24 hours) for estimation of percentage of unsealed and cleaned cells.

These preliminary results indicate high relevance of different environmental conditions on expression of pointed ethological traits in the tested honey bee populations. Also, there is a strong indication of wide range of interactions between mentioned conditions and the tested genotypes.

Results from the microsatellite analysis of honey bees used in GEI Experiment

Per Kryger[1]*, Marina Meixner[2], Maria Bouga[3], Roy Francis[1]

[1] Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark
[2] LLH-Bieneninstitut Kirchhain, Erlenstrasse 9, 35274 Kirchhain, Germany
[3] Lab of Agricultural Zoology & Entomology, Agricultural University of Athens, Athens, Greece

*per.kryger@agrsci.dk

One of the main goals of COLOSS WG 4 is to establish a common protocol for the discrimination of honey bee populations.

Honey bees, used in the common GEI experiment, were analyzed, using 24 microsatellite loci. DNA was extracted from the thoracic muscles of each bee, subjected to multiplex PCR and subsequent allele scoring methods. The results were compared with the existing data base in Aarhus University that represents the various subspecies used in the common experiment.

From the original 371 bees received for genetic analysis, samples that yielded less than 50% microsatellite data were removed from further analysis. Data from the remaining 317 individual bees were compared to the reference populations. The genetic profile of each bee was compared using multivariate methods (PCA, DAPC), as well as assignment tests in STRUCTURE and GeneClass.

DNA microsatellite data will be combined with these obtained from classical and geometrics morphometrics, alloenzymic analyses, as well as with these from mitochondrial DNA analysis in order to compare the various methods.

Contribution to bee Book and GEI experiment

Yves Le Conte*

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

*leconte@avignon.inra.fr

Two different sites are use 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' team. I will present the update of this experiment, including differences in survival rate, development and behavior of the different stocks.

Survival of honey bee colonies of the GEI experiment in Greece as a result of Varroa prevalence

Fani Hatjina*, Leonidas Charistos

Hellenic Institute of Apiculture (Hel.Agr.Org. "Demeter"), N. Moudania, 63 200 , Greece

*fhatjina@instmelissocomias.gr

The survival of the honey bee colonies of the GEI experiment in Greece was largely determined by the infestation of varroa mites. Since the beginning of the experiment, the infestation level were very low but just after the increase of the colonies during summer the infestation level was increased accordingly, and as a result a high number of colonies was lost during autumn 2010. The 10% infestation is considered the varroa threshold level, but exceptions exist in the rule.

Interestingly, the surviving colonies were entering the winter with very low infestation which was increased again after the summer increase of the colonies. Of the four groups tested, LigIT, CarVEIT and MacBUL lost most of their colonies, and only the local population, MacGR performed well with 7 of the 9 initial colonies still alive, while only 3 colonies of the rest populations are still alive. No brood removal was applied to any of the groups. During 20010 and 2011 summer periods. However, as infestation levels are currently very high (>10%), it is very interesting to see the survival of the colonies showing a kind of resistance till now.

Colony management on testing apiary

Nikola Kezić[1]* Maja Drazic[1], Janja Filipi[3] , Stefan Berg[4]

[1]Faculty of Agriculture University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia, [2]Croatian agricultural agency, Ilica 101, 10000 Zagreb, Croatia
[3]The University of Applied Sciences Marko Marulic, Knin, Croatia
[4] Das Fachzentrum Bienen der bayerischen Landesanstalt für Weinbau und Gartenbau, 97209 Veitshöchheim, Germany

*nkezic@agr.hr

Colony management is very various, and depends on the beekeeper or person responsible for the apiary. Colony management in testing apiary can significantly influence final results of the test. The basic request in establishing and later in maintaining colonies is to enable similar (same) conditions for all colonies on the test apiary. It is recommended to use standard (most common) hives for the test. It is not recommended to use queen excluder. Enough space needs to be given for each colony in proportion to its individual development. Hives should be placed on the stands that enable easy access to each colony and to reduce drifting of bees.

Water supply must be managed independently from the situation in the surrounding of the apiary and has to secure continues supply of pathogen free water. It is recommended to use wax foundation (free of residues), package bees and test queens for colony establishing. In the first weeks after establishing, colonies need to be fed with the same food in similar quantity at the same time.

Stabilizing the Varroosis parasite host system in beekeeping practice. 1. A concept to mimic natural live or die selection without colony losses

Stefan Fuchs[1]*, Cakmak Uludag[2]

[1]Institut für Bienenkunde Polytechnische Gesellschaft Goethe Universität Frankfurt am Main Karl von Frisch Weg 2 61440 Oberursel, Germany
[2]Ibrahim University M. Kemalpasa MYO Beekeeping Development and Research Center 16500, Bursa, Turkey

*s.fuchs@bio.uni-frankfurt.de

Selection is acting for increased tolerance in the Varroosis parasite-host system under natural conditions, which is apparent from observations of better balanced conditions at some locations, as well as from "live or die" experiments. However, as a rule these developments are accompanied by intolerable breakdowns of bee populations. Current treatment practice confers a short term advantage of preventing colony losses but offsets any long term advantages of better adapted genotypes spreading. We here propose a schedule of infestation dependent treatment and requeening to be implemented in ordinary beekeeping routines, mimicking natural selection processes.

DNA bank establishment from *Apis mellifera* populations inhabiting the territory of Bulgaria

Evgeniya Neshova Ivanova*, Stanimila Romanova Nikolova

Plovdiv University "Paisii Hilendarski" Biological Faculty,
Department of Developmental Biology, 24, Tsar Assen Str. Plovdiv 4000 Bulgaria

*geneiv@uni-plovdiv.bg

Creating of a DNA bank with genetic material samples of the Bulgarian *A. mellifera* populations is a science project stage with a real perspective for enrichment and development. Its goal is to have a centralized storage facility with DNA material and information,which can be used for future studies and research on a broad scale.The collected DNA could give and have information for the genetic history of the species, its populations and the complicated genetic relationships between and within them.

For the Bulgarian honey bees DNA bank establishment, up to this point have been used 1443 individual DNA samples.They inhabit 26 different populations on the country's territory.

That kind of an DNA bank with such examples will give a posibilityes to study the genetic variability among the honey bee populations in the country, which is important for their selection and conservation. Furthermore – this dives possibilities for studying the phylogenetic relationship between Bulgarian honey bees and other races and ecotypes from different regions of Europa with the idea of characterizing and being clear about the subspiecies status on the breeded honey bees in Bulgaria.

The individual DNA samples are keep in 2 ml Cryovial tubes and each tube has a linear barcode with individual information about DNA quantity, the concrete bee individual and its origin, the population and the region.

Till the moment, honeybee DNA samples were used for microsatellite DNA analysis of nine microsatellite loci: Ac011; A024; A043; A088; Ap226; Ap238; Ap243; Ap249 and Ap256.

The experience presented here could be used by COLOSS WG4 as idea to create a centralized European

Organization of breeding activity in Italy and coordinated genetic evaluation: limits and perspectives

Cecilia Costa*

CRA-API (Council for Research and Experimentation in Agriculture – Honey bee and Silkworm Research Unit) Via di Saliceto, 80 – 40128 – Bologna (Italy) +39 051 353103

*cecilia.costa@entecra.it

Beekeeping and queen breeding require no specific license in Italy, as long as the operation is registered with the health authority. Also, according to EU Reg. .... no impositions can be made concerning the subspecies of *Apis mellifera*. Italy is home to one of the most commercially important subspecies of honey bee, *A. m. ligustica*, currently distributed world wide, and to a phylogenetically diverse subspecies which evolved on the island of Sicily, *A. m. siciliana*, now close to extinction. To protect the autochtonous bees, and to provide the breeders with scientific support, a National Register was set up by the Ministry of Agriculture, which now counts ~ 30 breeders producing ~ 70,000 queens per year. CRA-API staff is in charge of coordinating breeding activities and organizing performance testing, as well as checking conformity to subspecies standards. Data is processed in the Institute in Hohen Neuendorf applying the modified BLUP model. The limit of applying this method to the Italian Ligustica queens is that mating is not severely controlled, nor is a single male line used. Validity of the model using only the maternal line will be discussed. Perspectives for the future include integration of vitality traits in the performance testing, application of methods derived from the COLOSS GEI experiment for local comparisons among strains, dissemination of importance of use of autochtonous subspecies.

Carnica ecotypes in different environments

Maja Drazic[1]*, Mateja Janeš[2], Janja Filipi[3], Dragan Bubalo[2],Nikola Kezić[2]
[1]Croatian agricultural agency, Ilica 101, 10000 Zagreb, Croatia,
[2]Faculty of Agriculture University of Zagreb, Svetosimunska 25, Zagreb, Croatia,
[3]The University of Applied Sciences Marko Marulic, Knin, Croatia

*mdrazic@hpa.hr

Carniolan bees (*Apis mellifera carnica*) are natively distributed from Central to the south and south east of Europe. Relocation of the honeybee subpopulations (ecotypes) into the new environment was not widely investigated. The objective of this experiment was to simultaneously compare colony development cycle and performance (honey yields) of three *A. m. carnica* subpopulations in two distinct environments in the native area. The study was carried out simultaneously at Pannonian region in Croatia (Mala Dapcevica) and Alps, Austria (Lunz am See). Test queen groups at each location contained 12 naturally mated *A. mellifera carnica* half-sisters originating from Austria (Institut Lunz am See), Slovenia (Agricultural institute of Slovenia) and from Croatia (Faculty of Agriculture Zagreb).

The dynamics of colony development was monitored every 14 days using transparent cellophane sheets (Bromenshenk and Lockwood-Ogan, 1990; modified method). At each measurement, every frame was taken out and for each side of the frame unsealed, sealed and drone brood, pollen and unsealed and sealed honey (in brood chamber) was drawn on the sheet. The surfaces of drawn areas were digitized by computer assisted image analysis. Honey yield per colony was calculated during honey extraction by weighing frames from honey suppers (without brood) prior and after extraction. At Mala Dapcevica location were 3 harvests (*Robinia pseudoacacia*, *Tillia* sp. and meadow), while in Lunz am See one (mix from spring to early summer). The statistical analysis was performed using the SAS. The differences the test colonies were observed between locations and ecotypes nested within location (sealed brood, pollen, honey yield).

Conservation of the Nordic Bee (*Apis mellifera mellifera*)

Lauri Ruottinen*, Juha Kantanen
MTT, Agrifood research Finland, 31600 Jokioinen, Finland

*lauri.ruottinen@mtt.fi

The Nordic Brown Bee (*Apis mellifera mellifera*) was earlier the most widespread bee in the world. It was present over large areas from the Alps to the Nordic countries and from the British Isles to Russia. Following expansion of its range, it has developed into a number of local, or at least geographically separate, lines.

The Nordic Brown Bee displayed several characters, which made it a relatively demanding breed to produce honey. There were practical difficulties to keep the Brown Bee: some colonies are very "runny" on the comb, relatively aggressive and defensive. Due to these less favourable chracters, the breed became replaced by other honey bee breeds, such as Buckfast, Caucasian, Italian and Carniolan Bee. The census size of the Nordic Brown Bee decreased also by crossing with other breeds. Currently, the Nordic Brown Bee does not have a commercial significance and organized breeding efforts have been finished. However, the breed does display excellent characters, such as significant winter hardiness, strong drive to collect pollen, high longevity of the worker bees and queen, and flight strength even in cold weather.

NordGen sent a 'small'questionnaire to Nordic and Baltic coordinators of Animal Genetic Resource Strategies in 2010 to collect basic data on the status of *A. m. mellifera*. The institutes coordinating the conservation activities of animal genetic resources in the Nordic countries are The Danish Plant Directorate, MTT Agrifood Research Finland, The Agricultural University of Iceland, The Norwegian Genetic Resource Centre, and The Swedish Board of Agriculture. This survey showed that *A. m. mellifera* has a threatened breed status and is included or at least mentioned in the Nordic Countries' national reports to FAO (State of the world on AnGR).

The Norwegian brown bee population is regarded as the largest in Europe and comprises 1500 colonies. In the Baltic countries there is a small brown bee population in Lithuania. There are some cross-border Nordic activities, including meetings and cooperation in the Laesö project. Conservation measures in Denmark, Norway and Sweden have been conducted.

Currently, there are Danish, Finnish, Norwegian and Swedish national bee-keepers' associations and local clubs which have activities in *A. m. mellifera* –conservation and have contacts to individual bee-keepers. On the European and even on the global level, a useful community to collaborate is the COLOSS –consortium (www.coloss.org).

As shown above, there are several activities to conserve and study *A. m. mellifera* but a proper networking and collaboration among the stakeholders are missing. The current project by NordGen in collaboration with MTT Agrifood Research Finland aims at collecting the current knowledge of the status of *A. m. mellifera* and activating networking and conservation of *A. m. mellifera* in the Nordic and Baltic countries.

Losses of bee colonies in the GEI experiment in Poland

Małgorzata Bieńkowska[1]*, Jerzy Wilde[2], Panasiuk Beata[1]
[1]Research Institute of Horticulture, Apiculture Division in Pulawy, Poland
[2]Apiculture Division University of Warmia and Mazury in Olsztyn, Poland

malgorzata.bienkowska@man.pulawy.pl

From 124 of experimental colonies established in the summer 2009 the average losses to the autumn 2010 reached 29%, but winter losses averaged 23.4% (from 0.00% to 71%). The highest losses were recorded in Kunki apiary (37.8%). Over 71% of *Apis mellifera mellifera* colonies did not survive until spring 2010. Until autumn 2011 only 20.1% of all bee colonies survived: in Kunki about 30%, and in Gąsiory and Bronowice respectively 19 and 14% of colonies.

Analysis of winter debris as well as bees collected from colonies which did not overwinter or died in early spring, showed that infection with *Nosema* spores and autumn infestation with *V.destructor* mite was significantly higher than in colonies that survived the winter. The exception in the first year of the study were colonies from car C, Mel P and Car L lines that were lost but the high mite infestation or nosema infection were not recorded.

It was found that until autumn 2011 winter bee losses averaged 18.5%. Losses caused by the parasitic *V. destructor* mite averaged for 27%, and as a result of colony weakness 13% of bee colonies were lost. One reason for the colony withdrawal from the experiment was weakening of bee colony due to queen loss or drone egg laying. These losses were 18.5%.