

## Action FA0803: Prevention of honeybee Colony Losses (COLOSS)

### Scientific Report of the Work Shop

**COST-FA0803-290709-05908**

### STANDARDIZATION OF METHODS II: Vitality testing

**Bee Institute Kirchhain, Germany, 28.07.-02.08.2009**

Time	Program
<b>28.07.2009 (Tuesday)</b>	
19:30	Arrival  Informal social gathering in the hotel "Bärenhof" in Stadtallendorf: The local organizers Dr. Büchler, Dr. Meixner and Ms. Heidinger were meeting together with the participants for a welcome reception and discussions.
<b>29.07.2009 (Wednesday)</b>	
8:00 - 8:45	Registration and welcome The participants were welcomed by the local organizer Dr. Büchler. The director of the agricultural state unit of Hessen (Landesbetrieb Landwirtschaft Hessen), Mr. Andreas Sandhäger, was present for the opening of the meeting and addressed a welcome note to the participants.
8:30 – 10:40	<p style="text-align: center;"><b>Standardization of colony performance and vitality tests: contributed talks I</b></p> <p>The session was chaired by Dr. Büchler.</p> <p>Dr. Kryger's talk reviewed several methods currently available for automated data recording of honey bee colony parameters, and discussed their potential use for vitality evaluation. He also briefly reviewed the influence of genetic origin on disease susceptibility.            In the discussion, the participants expressed great interest in automated Nosema spore counts and Varroa detection.</p> <p>The presentation of Dr. Panasiuk summarized a comparison of different breeding lines that were tested for hygienic behavior using different test methods (pin test, freezing, and inoculation with <i>Ascosphaera apis</i> spores). A lively discussion followed, focusing on the methodological aspects and relationship between hygienic behavior and disease susceptibility.</p> <p>Dr. Wilde presented a comparison between different methods of assessing Varroa infestation levels in a sample of adult bees. The methods discussed appeared equally well suited to this purpose.</p>



	<p>After describing the methods used in her lab for the measurement of population and colony strength, Dr. Hatjina presented results of experiments with different concentrations and formulations of oxalic acid to treat against <i>Varroa destructor</i> in a climate where brood is present all year round. She also discussed a possible toxic effect to honey bee larvae and the presence of residues in hive products.</p> <p>Dr. Dahle gave an overview of beekeeping and breeding in Norway, including a description of traits used for selection. Although some areas in Norway still appear to be free of <i>Varroa</i> mites, the start of a selection program incorporating <i>Varroa</i> resistance parameters is anticipated. The estimation of breeding values according to the system used in other parts of Europe is planned.</p>
11:00-13:00	<p style="text-align: center;"><b>Standardization of colony performance and vitality test: contributed talks II and contributed posters</b></p> <p>The session was chaired by Dr. Kryger.</p> <p>The objective of the project presented by Dr. Garrido was to develop criteria that are useful indicators of survival of a honey bee colony under parasite stress from <i>Varroa destructor</i>, including the development of detection methods feasible for use by beekeepers. She also discussed the establishment of a “vitality test” as an additional selection tool and the predictive values of colony development and mite infestation rate for colony survival.</p> <p>The concept of the survival test and the development of threshold values indicating survival probability were discussed among the participants.</p> <p>The talk of Dr. Kezic illustrated the fact that the activity of queen breeding is new to Croatia, since traditionally queens used to be imported from Slovenia. An official program of bee breeding was started in 1997. He also discussed the reluctance of queen breeders to participate in disease tolerance selection due to the perception of beekeepers who expect to receive bees from such a program that never need to be treated.</p> <p>A lively discussion about this particular aspect followed, since several participants of the workshop have made similar experiences.</p> <p>In her talk, Cecilia Costa reviewed different aspects of using hygienic behavior as a selection criterion that were first discussed among WG4 at the Athens meeting. She gave an overview of the methods available and the documented relations of this trait to disease resistance. She also presented a synopsis of the advantages and disadvantages of each method and the caveats to be observed in their practical use.</p> <p>This subject was intensified in the following discussion, when Dr. Büchler briefly presented unpublished data from previous experiments conducted at the Kirchhain institute comparing different methods of hygienic testing (pin test, freeze kill, and Jenter test) together with their repeatabilities.</p> <p>Three contributed posters were discussed in the poster session before the lunch break:</p> <p>Ivanova and Petrov: Inventory of the races of honey bees belonging to</p>

	<p>different bio-geographical regions in Bulgaria based on morpho-ethological, population genetic and productivity characteristics.</p> <p>Petrov et al.: Influence of season and degree of <i>Varroa destructor</i> contamination on local Bulgarian honeybees.</p> <p>Nanetti et al.: Climate change and apiculture – impacts on wellbeing and survival of bee colonies.</p> <p>Bienkowska: Daily summer fall of <i>Varroa destructor</i> calculated from short sampling periods to be used as an indicator of autumn mite infestation of honey bee colonies.</p>
13:00-14:00	Lunch
14:00 - 18:00	<p style="text-align: center;"><b>Field training: colony performance testing and biotechnical control methods</b></p> <p>In the afternoon, various aspects of practical field testing were demonstrated and discussed. The participants were taken by car to three different apiaries in the vicinity of Kirchhain: (1) “Wäldershausen Tal”, (2) “Homberg”, and (3) “Lahnberge”.</p> <p>In apiary 1, the general methods of performance testing practiced at the Kirchhain institute were reviewed and discussed, including record keeping and the annual management regime. The colonies in this apiary had been subjected to complete brood removal as treatment against <i>Varroa</i> mites some weeks earlier. The concept and practical application of this treatment option was discussed on site. Another important activity at this apiary was the demonstration of the pin test as one method of testing for hygienic behaviour. The method was shown in detail several times and the participants had the opportunity to actively practice it themselves.</p> <p>The colonies in apiary 2 had been assembled from the brood combs removed from those treated colonies in the beginning of July. Here, we discussed sampling methods for detecting the level of <i>Varroa</i> mite infestation in bee colonies together with practical demonstrations.</p> <p>Apiary 3 consisted of colonies that had been established recently in preparation for a new cycle of performance testing starting this fall. The methods to set up, equalize and prepare colonies for a new test cycle were demonstrated and discussed.</p>
19:00	Visit to Marburg and dinner at the restaurant “Bückingsgarten”
<b>30.07.2009 (Thursday)</b>	
8:00 – 10:45	<p style="text-align: center;"><b>Genotype-environment interactions on bee vitality: contributed talks III</b></p> <p>The session was chaired by Ms. Costa</p> <p>Dr. Kence gave a presentation on variation of the expression of hygienic behavior in three different honey bee subspecies native to Turkey. The test was conducted both in Ankara and in the respective natural locations of each subspecies. One of her conclusions was that the “performance” of hygienic behavior was better in the native range.</p> <p>Aspects of the methodology used were discussed. Another subject of the discussion was the impact of commercial queen breeding and distribution</p>

	<p>on the natural honey bee population of a region.</p> <p>Dr. Nedic referred about queen breeding activities in Serbia and ecotypes of <i>A. m. carnica</i> that may be present there. Bees from several different regions of Serbia were brought to Belgrade and compared, regarding several aspects of apicultural interest. The results provide the basis for further breeding activities using local varieties.</p> <p>Dr. Bouga's presentation was given by Per Kryger, since she had to cancel her participation on short notice. She proposed the development of a method of standardizing the certification of origin of a given honey bee queen or colony, since different approaches are currently in use in Europe. A very active discussion followed. In general, there was consensus about the necessity of standardization. It was proposed to use a combination of methods, preferably including (geometric) morphometric methods and DNA methods (microsatellites). It was also agreed that there is an urgent need to create accessible reference data for European honey bee populations. The methodological and technical details will be discussed by a smaller working group who will also propose methods and develop protocols (Meixner, Kryger, Costa, Hatjina, Bouga, Bienkowska, Panasiuk, Kence, Nedic). The aim is the creation of a published and accessible reference data base.</p> <p>Dr. Korpela presented a talk about tracheal mite infestations in Finland and possible control measures against this parasite. Since the effectivity of chemical treatments appears limited, he discussed the potential benefits of incorporating resistant bees from Macedonia into selection programs.</p> <p>In his talk about winter food consumption of <i>A. m. macedonica</i>, Mr. Uzunov discussed the adaptation of honey bee management concepts to the regional conditions of southeastern Europe.</p> <p>Dr. Celle presented a talk about the aims and scope of the experimental centre of honey bees in Toulouse. The centre has been set up both as a means of networking among the beekeeping community and as a source of genetic stock material for beekeepers.</p> <p>Mr. Maori talked about vitality assays with bees affected by viruses, on the levels of cages, reproductive nucs and full-sized colonies</p>
10:45 - 11:00	Coffee break
11:00- 13:30	<p><b>Discussion of a common test protocol</b></p> <p>The session was chaired by Dr. Meixner  Discussion of genotype-environment experiment:  The participants updated the genotype allocation list and a detailed discussion of a standardized common test protocol followed. The discussion was continued after the lunch break and the laboratory demonstrations.</p>
13:30 -14:00	Lunch
14:00 - 15:00	<p><b>Laboratory demonstrations and practice</b></p> <p>Practical demonstrations of ongoing laboratory activities were given. A method for assessing mite infestation in samples of adult bees was</p>

	demonstrated and practiced by the participants. In addition, molecular biological techniques for the diagnosis of viral bee diseases and techniques for instrumental insemination of queens were demonstrated.
15:00 – 16:15	<p style="text-align: center;"><b>Testing and breeding for colony vitality: discussion of international recommendations (continued)</b></p> <p>Open discussion chaired by Dr. Meixner: Very productive discussions on vitality test organization and goals. A final wrap up discussion summarized the main issues of the Work Shop:</p> <ul style="list-style-type: none"> <li>A) The details of the genotype-environment experiment were finalized. A total of 760 colonies belonging to 19 different genotypes will be distributed among 17 participants of the experiment. The experimental colonies will be managed and evaluated according to a common test protocol until the spring of 2012. Details of the methodology were determined. An experiment record card common to all participants will be used. It was agreed to use a method measuring the percentage of brood area on each comb for assessment of colony strength. For the test of hygienic behavior, it was agreed to use the pin test.</li> <li>B) Standardization of methods to assess the level of Varroa infestation within the experiment was discussed and will be adapted to the respective local conditions of each participant. The experiment will contribute to determine regional threshold levels for Varroa infestation.</li> <li>C) Based on the results of this discussion, international breeding recommendations including vitality tests will be designed, consisting of both the theoretical framework and technical and methodological aspects. A small working group within WG4 was formed to draft a first version of the recommendations. At least the technical and methodological aspects will be available until March of 2010.</li> <li>D) It was agreed that there is an urgent need to create accessible reference data for European honey bee populations. The methodological and technical details will be discussed by a smaller working group who will also propose methods and develop protocols. The aim is the creation of a published and accessible reference data base.</li> <li>E) The next workshop “Standardization of methods III: vitality test” is envisioned for the spring or summer of 2010.</li> </ul>
16:15 – 19:00	<p style="text-align: center;"><b>Field training - vitality and survival testing of colonies</b></p> <p>The participants were taken by car to an apiary where colonies for the genotype-environment interaction experiment have been set up. Different methods of estimation and measurement of adult bee population and brood extension were demonstrated: 2 percentage methods, use of 2 different counting grids for bees or for brood, estimation of bee numbers and measurement of brood surface. For each one of the methods a training session took place, and results of the different tests and participants were compared.</p>
19:30	Dinner at the restaurant “Brücker Mühle” in Amöneburg
<b>31.07.2009 (Friday)</b>	

8:00 - 10:30	Transfer to Schwarzenau
10:30 - 13:00	<p>Visit of the Bavarian colony test station "Schwarzenau".</p> <p>Dr. Berg illustrated the Bavarian State breeding program, whereby performance testing is compulsory by law for queen breeders selling &gt;50 queen bees per year. He reported that groups of sister queens are distributed in three climatically different testing areas and evaluated according to productivity and vitality traits.</p> <p>Modern equipment used for assessing hive development and environmental factors was demonstrated.</p> <p>Methods for assessing colony strength were further practised using the colonies in the testing station; the results obtained using three methods simultaneously on three different colonies were compared. It was decided that estimation of population sized is suitable for assessing the adult bee population, while measurement is more reliable for quantity and kind of brood.</p>
13:00 - 14:30	Lunch
14:30 - 17:30	<p>Transfer to Veitshöchheim, visit of the bee institute in the "Bayerische Landesanstalt für Weinbau and Gartenbau"</p> <p>Dr. Illies presented the Bavarian Institute for Viniculture and Horticulture, of which the Honey Bee Institute is part, and described the extension services of the unit. Dr. Berg further presented the ongoing research of the bee unit, including the development of a test for correlation between hygienic behaviour and removal of varroa-infested brood by use of a wax comb placed onto a sheet of plexiglass. A project on effects of different pollen diets was presented by a student working at the Unit.</p>
17:30 - 19:00	Transfer to Triesdorf, dinner in a local restaurant
<b>01.08.2009 (Saturday)</b>	
09:00 – 12:30	Meeting of the national breeder cooperation "Arbeitsgemeinschaft Toleranzzucht" (network of bee breeders, beekeepers and institutes working towards selection for disease resistance), including a visit of the local agricultural training centre "Triesdorf".
12:30 – 13:30	Lunch
14:00 – 16:30	Demonstrations at a drone congregation area and visit to the queen breeding station of the agricultural training centre "Triesdorf".
17:00 - 21:00	Return to Kirchhain or Frankfurt/Main (Airport)
<b>02.08.2009 (Sunday)</b>	
	Departure

## Appendix 1:

### List of attendants

1	Berg	Stefan	Germany
2	Bienkowska	Malgorzata	Poland
3	Büchler	Ralph	Germany
4	Celle	Olivier	France
5	Costa	Cecilia	Italy
6	Dahle	Bjørn	Norway
7	Dyrba	Winfried	Germany
8	Garrido	Claudia	Germany
9	Hatjina	Fani	Greece
10	Heidinger	Ina	Germany
11	Ivanova	Evgeniya	Bulgaria
12	Kence	Meral	Turkey
13	Kezic	Nikola	Croatia
14	Korpela	Seppo	Finland
15	Kryger	Per	Denmark
16	Maori	Eyal	Israel
17	Meixner	Marina	Germany
18	Nanetti	Antonio	Italy
19	Nedić	Nebojša	Serbia
20	Panasiuk	Beata	Poland
21	Petrov	Plamen	Bulgaria
22	Ruottinen	Lauri	Finland
23	Uzunov	Aleksandar	Republic of Macedonia
24	Wilde	Jerzy	Poland