



Action FA0803



**Proceedings of the
COLOSS Workshop
STANDARDIZATION OF METHODS II:
VITALITY TESTING**

**Bee institute Kirchhain
28. July - 02. August 2009
Kirchhain, Germany**

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Agenda

| TIME | PROGRAMME |
|---|---|
| 28.07. 2009 (Tuesday) | |
| | Arrival to Kirchhain |
| 19:30 | Welcome and social gathering |
| 29.07.2009 (Wednesday) | |
| 8:00 - 8:30 | Registration |
| 8:30 - 9:00 | Welcome and introduction (Ralph Büchler) |
| Standardization of colony performance and vitality tests: contributed talks I | |
| 9:00 - 9:20 | New tools for valuation of vitality characters (Per Kryger) |
| 9:20 - 9:40 | Testing honey bees for hygienic behaviour (Beata Panasiuk) |
| 9:40 - 10:00 | Detection of Varroa mite with use of sugar powder (Jerzy Wilde) |
| 10:00 - 10:20 | Estimating brood area and bee population in a honey bee colony-control for Varroa infestations (Fani Hatjina) |
| 10:20 - 10:40 | Performance testing in the national bee breeding program in Norway (Bjørn Dahle) |
| 10:40 - 11:10 | Coffee break |
| Standardization of colony performance and vitality test: contributed talks II and contributed posters | |
| 11:10 - 11:30 | Establishing vitality tests as a new selection tool (Claudia Garrido) |
| 11:30 - 11:50 | Queen breeder and Vitality test (Nikola Kezic) |
| 11:50 - 12:10 | Hygienic behaviour tests (Cecilia Costa) |
| 12:10 - 13:00 | Contributed posters and final discussion |
| 13:00 - 14:00 | Lunch |
| 14:00 - 18:00 | Field training: colony performance testing and biotechnical control methods |
| 30.07.2009 (Thursday) | |
| Genotype-environment interactions on bee vitality: contributed talks, discussion of a common test protocol | |
| 8:00 - 8:20 | Variation in grooming and hygienic behaviors in three honey bee races found in Turkey (<i>Apis mellifera caucasica</i> , <i>A. m. syriaca</i> , <i>A. m. carnica</i>) (Meral Kence) |
| 8:20 - 8:40 | Hygienic behaviour of some selected honey bee (<i>Apis mellifera carnica</i>) lines in Serbia – methods and results (Nebojša Nedić) |
| 8:40 - 9:00 | Comparative test on vitality in various locations: certification of honey bees' origin (Maria Bouga) |
| 9:00 - 9:20 | Adaptation and performance of Greek honey bees resistant to <i>Acarapis woodi</i> in Finland (Seppo Korpela) |
| 9:20 - 9:40 | Overwintering food consumption on the testing apiary in republic of macedonia (Aleksandar Uzunov) |
| 9:40 - 10:00 | Presentation of the experimental centre of honey bees in Toulouse (Olivier Celle) |

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|------------------------------|--|
| 10:00 - 10:20 | Recruiting colony vitality assays for evaluation of virus virulence as well as viral disease control: the case of Israel Acute Paralysis Virus (IAPV) (Eyal Maori) |
| 10:20 - 10:50 | Coffee break |
| 10:50 - 11:20 | Discussion of a common test protocol (Ralph Buechler <i>et al.</i>) |
| 11:20 - 13:00 | Testing and breeding for colony vitality: discussion of international recommendations |
| 13:00 - 14:00 | Lunch |
| 14:00 - 18:00 | Field - and laboratory training: vitality and survival testing of colonies |
| 31.07.2009 (Friday) | |
| 8:00 - 10:30 | Transfer to Schwarzenau |
| 10:30 - 13:00 | Visit of the Bavarian colony test station "Schwarzenau" |
| 13:00 - 14:30 | Lunch |
| 14:30 - 17:30 | Visit of the Bavarian bee institute "Veitshöchheim" |
| 17:30 - 19:00 | Transfer to Triesdorf |
| 01.08.2009 (Saturday) | |
| 09:00 - 17:00 | Representation of the national breeder cooperation "Arbeitsgemeinschaft Toleranzzucht" including a visit of the local training centre "Triesdorf" and demonstration at a drone congregation area |
| 17:00 - 21:00 | Return to Kirchhain or Frankfurt/Main (Airport) |
| 02.08.2009 (Sunday) | |
| | Departure |

Oral Presentations

Comparative test on vitality in various locations: certification of honey bees' origin.

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According the experimental design of comparative test on vitality in various locations of COLOSS Working Group 4:

“At each test location one locally adapted strain (autochthonous subspecies or traditionally kept bee race), will be compared to at least two strange origins. Each participating partner is responsible of certifying the declared origin. Samples of workers from each test colony will be collected during the experiment for a possible future genetic analysis”.

The certification of bee's subspecies is very important but not yet standardised. It is known that different certification systems are applied in several countries, based on different methodology: classical morphometrics (but the characters that are measured are not always the same: cubital index is the most common character that is used), geometric morphometrics (3 different methods), molecular markers (isoenzymes, mitochondrial DNA, microsatellites etc). The problem of mixture of honey bee subspecies due to beekeeping manipulations and commercial breeding, effects on the certainty of detection of honey bee subspecies. There is the necessity to develop a common protocol for the certification of honey bee origin. In order not to have delay of the experimental process of the comparative test on bee vitality, this common protocol can be developed in parallel with the method that is already used in each country. In the end of the experimental process of the vitality test, comparable results and a common genetic data base concerning honey bee's origin will be obtained.

Vitality testing and selection for *Varroa* resistance in the Norwegian national honeybee breeding program

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Even though *Varroa destructor* is associated with somewhat higher rate of colony losses in winter (10.3% vs 6.3% in areas without *Varroa*), beekeepers in Norway does not find *Varroa* as a major threat to their bees as long as they follows the guidelines for *Varroa* treatment given by the Norwegian Beekeepers Association. This treatment includes trickling with oxalic acid in fall when the colonies are broodless and cutting sealed dronebrood in spring and summer. Some beekeepers additionally use formic acid in summer after harvesting the summer honey. The reason for the limited problems with *Varroa* in Norway might be related to a long broodless period limiting the reproduction of the mite, low density of colonies and well organized beekeepers that follows the recommended treatment procedures, thus limiting the reinvasion of *Varroa* from neighbouring beekeepers, and less problems with secondary virus infections, although nearly nothing is at present known about the presence of different viruses in honeybees in Norway. Well aware that the problems with *Varroa* might increase in the future, it is important to include *Varroa* resistance in the national breeding program for honeybees. As breeding values from now on will be calculated using the genetic evaluation at the Institute of Bee Research in Hohen Neuendorf, we will probably use the same methods to measure *Varroa* resistance. Breeding for *Varroa* resistance, in which hygienic behaviour is an important component, might indirectly select for resistance towards chalk brood (*Ascosphaera apis*) which seems to be an increasing problem. The inclusion of the Norwegian strains of *mellifera* and *carnica* (the latter imported from Austria in the late 1970ies) into the vitality testing would be valuable. However this would be difficult due to import restrictions on queens, and at present limited testing capacity. However we are planning to conduct vitality tests of the strains in Norway at a national level according to the methods and protocols developed by COLOSS Working Group 4.

Establishing vitality tests as a new selection tool

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During a four years lasting Varroosis tolerance breeding project vitality tests were introduced. At the beginning, we intended to test the relevance of Varroa infestation development and brood hygienic behaviour for the survival without varroa treatment. After the first winter, the data indicated the possibility to use the viability tests as a new selection tool.

To overwinter bee colonies without treatment against varroosis within a breeding program it was necessary to find criteria to predict the probability of survival. Bee colonies surviving or dying in the following winter differed significantly in Varroa infestation, colony strength and frequency of virus infections already in late summer. These parameters may be used for choosing bee colonies for these vitality tests.

In addition to simple survival without treatments against varroosis, the overwintering performance may be taken as a parameter for selection. The quotient of colony strength before and after winter (overwintering index) is an indicator for the loss of bee mass during the winter. Only those colonies with highest overwintering indexes should be considered for further breeding purposes.

Variation in grooming and hygienic behaviors in three honey bee races found in Turkey (*Apis mellifera caucasica*, *A. m. syriaca*, *A. m. carnica*)

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Hygienic behaviour is a genetically controlled trait which provides resistance to diseases in honey bee colonies. We evaluated the hygienic behaviour of three races, *Apis mellifera caucasica*, *A. m. carnica*, and *A. m. syriaca* from Artvin, Kirklareli, and Hatay respectively. Detection of hygienic behaviour was carried out by observing the removal rate of freeze-killed brood from the colonies at two different locations, at METU bee common garden and their hometowns. Bees from Kirklareli (*carnica*) and Hatay (*syriaca*) are found more hygienic than Artvin (*caucasica*) when kept at METU in Ankara, however Artvin bees were much better in hygienic behaviour at their hometown. Dead brood removal rate measured as 28% in Ankara whereas 66% at original location for Artvin, for Kirklareli the rates were 50% and 62%, higher value was at the hometown. These observations suggest that although there is genetic variation among the races, environmental conditions also affect the hygienic behaviour. Grooming against *Varroa* was also studied and found to be the highest (100%) in African bees. It was also high in *caucasica* (Artvin) bees compared to that of the *syriaca* (Hatay) and *carnica* (Kirklareli) which may be explained that they are kept at an isolated area where no treatment against *Varroa* applied. Thus they were on their own to combat with *Varroa* and they probably developed resistance in adapting to live with that pest by increased grooming behaviour

Estimating brood area and bee population in a honey bee colony- control for Varroa infestations

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For several years we used to estimate brood area using a frame divided into 2x2cm squares (4cm²) with nylon strips. One frame contains 21 x 10 (=210) such squares. The measurement is very accurate and the method is easy and quick. Bee population can also be easily measured by using a similar frame divided in larger squares (6x6cm). In one Langstroth frame there are 7 x 3 (=21) such large squares, each containing about 50 bees. Oxalic Acid dyhydrate is used for winter and summer control of Varroa. For winter control on queenless colonies we use one application of 80g of OA diluted in sugar syrup made from 1lit of water and 1kg of sugar (3.5% of OA w/v). However, during summer, lower concentration of OA is used (60g) in a more diluted sugar solution made from 1lit of water and 500g of sugar (3.2% of OA w/v). Summer applications with the above dosage (one or two) have no effect on brood production, population, queen status or honey residue levels.

Adaptation and performance of Greek honeybees resistant to *Acarapis woodi* in Finland

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It is known that *Apis mellifera macedonica* (honey bees from North Greece) is resistant to *Acarapis woodi*. During 2002, the prothoracic spiracles of three honey bee strains used commercially in Greece (from N. Greece, N. Italy and Slovenia) were examined with a light microscope in order to detect possible morphological differences between the strains as an attempt to explain the resistance showing by the Greek Macedonian (N. Greece) strain but not by the others against *Acarapis woodi* Rennie. Certain dimensions, like the length and the area of aperture in the prothoracic spiracle, were found to be significantly larger in the honey bees from N. Italy and Slovenia compared with the honey bees from N. Greece. The fact that the prothoracic spiracle of the honey bees from N. Greece was significantly smaller, compared with the other honey bee groups (from N. Italy and Slovenia), could be a possible mechanism for resistance developed by this strain against *Acarapis woodi* R. (Hatjina *et al.*, 2004).

In order to know if *Apis m. macedonica* will continue to be resistant in Finland, where tracheal mite disease is a problem, 10 sister macedonian queens were shipped to Finland in the summer of 2008. The queens were established in full size colonies but only 5 of them managed to over-winter. The experiment is in progress and observation will be done during summer 2009.

The identification of *Apis m. macedonica* is based on RFLP's method, using StyI and NcoI restricted enzymes that recognize one site on COI gene segment of mtDNA, only in this subspecies (Bouga *et al.*, 2005a). This method can be applied directly to queens, using non-lethal method (Bouga *et al.*, 2005b).

Hatjina, F., Gregorc, A., Papaefthimiou, C., Pappas, N., Zacharioudakis, S., Thrasyvoulou, A., Theophilidis, G. (2004) Differences in the morphology of prothoracic and propodeal spiracles in three strains of *Apis mellifera*: possible relation to resistance against *Acarapis woodi*. *Journal of Apicultural Research* 43(3): 105 – 113.

Bouga, M; Harizanis, P C; Kiliadis, G; Alahiotis, S (2005a) Genetic divergence and phylogenetic relationships of Honey Bee *Apis mellifera* (Hymenoptera: Apidae) populations from Greece and Cyprus using PCR - RFLP analysis of three mtDNA Segments. *Apidologie* 36: 335-344.

M. Bouga, E. Klossa-Kilia, S. Alahiotis and G. Kiliadis (2005b) Non-lethal DNA sampling of wing tips discriminates subspecies of *Apis mellifera* occurring in Greece. *Journal of Apicultural Research* 44(4): 195-196.

New tools for evaluation of vitality characters

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The study of honey bee vitality involves many factors. Quantitative measurement of these factors can be time consuming. I present some of the methods that we have tried developing in Denmark to facilitate recording and increase data amounts. The Danish beekeepers have a long tradition for digital health control. An image analysis program was developed in the 1980ies to count Nosema spores. The results from these Nosema counts have been used for more that 20 years for selective breeding. This has reduced the occurrence of Nosema in Danish bee breeders' stock to less than 20 % of the colonies. We believe that counting varroa mites on debris or washed from bees is another method ready for image analysis.

At the University of Aarhus, we have developed scale hives to improve our knowledge of yearly fluctuation, based on colony weight measured every minute. We aim to combine this with bee counters that can measure the movement of bees in and out of the hive. Increased loss of bees would indicate impaired vitality.

One method is still evading us, the automatic recognition of brood, pollen, nectar, and honey cells from digital images. We continue working on that, as it would improve our knowledge of colony health considerably.

An additional factor in hive health is the genetic diversity of colonies. We have been studying a subset of colonies with symptoms of American foulbrood. Alarmingly, we found high levels of inbreeding in these colonies, the queen mating with closely related drones, due to breeding in closed populations on isolated islands. We consider a causal relation. We intend to increase our sample size in the coming months based on these first indications.

Recruiting colony vitality assays for evaluation of virus virulence as well as viral disease control: the case of Israel Acute Paralysis Virus (IAPV)

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Bee colony losses, in particular Colony Collapse Disorder (CCD), have become a major global economic concern since bees are the most common pollinators of many food crops. IAPV is a bee-affecting dicistrovirus which has been strongly associated with CCD. Investigation of IAPV and its honeybee host dynamics required the development of several host vitality assays in order to evaluate virus virulence on the one hand and bee resistance to viral infection on the other. Since IAPV-honeybee interactions were studied on a wide range of colony scales (from plastic boxes through mini-hives to standard hives), the reported vitality assays were adapted to the colony size and production as well as to the time period of the experiment. Vitality assays of virus-affected colonies will be demonstrated and the potential use of these methods as a general tool for testing colony-pathogen dynamics will be discussed.

The methods of estimation bee and brood population of honey bee (*Apis mellifera carnica*) in Serbia

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For apicultural practice the productive traits of bee colonies are of great significance, since it is related to different geographical bee breeds. For the purpose of preservation of existing populations and natural diversity of honey bee and for the purpose of their further selection, researches upon the productive features of certain individual populations of choice at the territory of Republic of Serbia.

The bee lines of the *Apis mellifera carnica* race from different geographical localities in the Republic of Serbia were included in the research. The research was carried out on the beehive of the different localities at apiaries through Serbia. Bee societies were put into standard Langstroth-Rut beehives and at the start of the trial their equalizing per strength was performed. Following productive traits were examined: the surfaces of bees, brood, honey and pollen. The evaluation was performed in two spring examinations (I and II) before the beginning of black locust pasture. The reading was conducted on the individual frame in 1/10 frame, by the method prescribed in the The Regulations on how to research the breeding livestock traits (Official Register of RS 21/96). The data were analyzed by a standard factorial variance analysis investigating the traits in different bee lines.

Testing honey bees for hygienic behaviour

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Hygienic behaviour of the honey bees is one of the mechanisms for disease resistance. It involves the detection of diseased or dead brood in sealed cells, uncapping, and removing it from the nest. The rate of cleaned cells depends on strength of bee colony, age of workers, natural flow and weather conditions. Studies on hygienic behaviour of bees were carried out in the Department of Bee Breeding, Apiculture Division in Pulawy, Poland in the years of 2004-2005. The aim of the research was to study response of bees to dead brood inserted into the colony. The number of cleaned cells with, freeze-killed, *Ascosphaera apis* inoculated and pin-killed brood was checked.

Significant differences were found in the rate of removing dead brood. In both years of the research pin-killed brood was significantly faster recognized and removed by bees. Significantly lower rate of cleaning cells with *A. apis* inoculated and freeze-killed brood was observed. The highest differences in cleaning rate were found within the first 12 hours of cells cleaning.

Hygienic behaviour of some selected honey bee (*Apis mellifera carnica*) lines in Serbia - methods and results

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Local honey bee ecotypes with higher vitality are very important in modern apiculture. Hygienic behaviour in honey bees is an behavioural mechanism of disease resistance. The Carniolan honey bee (*Apis mellifera carnica*) colonies placed in LR beehives with 10 frames have been tested for hygienic behaviour, as a possible basis for resistance to mites and hence the basis for selection and breeding of varroa resistant domestic honey bees. Hygienic behaviour was studied in three selected lines of honey bees (1-K, 2-V and 3-S) in two intervals of 24 and 48 hours. We slightly modified and accelerate the implementation of standard pin-killed method by constructing especially gadget with pattern of 50 and 100 fixed entomological pins. Results revealed extremely variable, but non-significant differences in hygienic behaviour between colonies in both times of measuring. Honey bee colonies from 3-S line, after 48 h, had highest level (86.85%), while line 2-V had lowest level of cleaned cells (79.67%). The further selection of highly hygienic colonies will be carried out at the colony level.

Overwintering food consumption on the testing apiary in Republic of Macedonia

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In order to determine the average colony amount of food consumption we have regularly measured 10 colonies in one apiary on monthly basis. The colonies (hives) were measured in total after which we have excluded the mass of the hive, frames, wire, wax and honey bees. Additionally, for estimation of the statistic correlation, the number of covered streets (inter-frames space) and daily maximum-minimum temperatures were measured. The experiment started in November, 2008 and finished in March, 2009

Additional Oral Presentations

- Presentation of the experimental centre of honey bees in Toulouse (Celle Olivier)
- Hygienic behaviour tests (Costa Cecilia)
- Queen breeder and Vitality test (Kezic Nikola)

Poster Presentations

Inventory of the race belonging of honey bees in different bio-geographical regions in Bulgaria based on morpho-ethological, population-genetic and productivity characteristics

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A new project for scientific and selective work with honey bees was supported by Ministry of Agriculture in Bulgaria for 2009/2010. The main purpose of the project is an inventory of the race belonging of honey bees in different bio-geographical regions in Bulgaria. The methodology planned to be used is based on a complex approach: classical and geometric morphometry, ethological, isoenzyme and molecular-genetic analysis. Till the moment different morphometrical characteristics for bee queens (length of proboscis (mm), forewing length (mm), forewing width (mm), cubital index, discoidal shifting (%), tarsal index (%), diameter of the spermatheca (mm), coloration), worker bees (length of proboscis (mm), length of the front wing forewing length (mm), width of the front wing forewing width (mm), cubital index, discoidal shifting (%), wax-mirror back border (%), length of the pappi of the 5-th abdominal tergum (mm), hair index of the 4-th abdominal tergum, total sum of the lengths of 3-rd and 4-rd abdominal tergum (mm), length of the hind leg (mm), tarsal index (%), coloration of the abdominal tergums, colour of the pappi of the 2-nd abdominal tergum, colour of the pappi of the 3-nd to 6-th abdominal tergums) and drones (length of proboscis (mm), length of the front wing forewing length (mm), width of the front wing forewing width (mm), cubital index, discoidal shifting (%), total sum of the lengths of 3-rd and 4-rd abdominal tergum (mm), coloration of the abdominal tergums, colour of the thorax pappi) are studied. Polymorphism on six different isoenzyme systems (EST, MDH, ME, PGM, HK, ALP) are characterized. Live weight (mg) of unfertilized and fertilized queens, density (capacity) of worker brood, average fecundity for twenty-four-hour period and the diameter of spermatheca are parameters used for characterization of queen quality. Ethological characterization includes hygienic behaviour, winter resistance, swarming inclination, aggressiveness level, honey and wax productivity.

Climate change and apiculture - Impacts on wellbeing and survival of bee colonies

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The Fourth Assessment Report of IPCC confirmed that greenhouse gas emissions from human activities have a real influence on the planet's climate. Because of rapid changes like temperature increase, irregular weather and drought also honey bee colonies are facing new problems. These are related to the complex interactions between their life cycle, climate anomalies, blossoming ages, pest and parasite development, control measures etc. As a consequence, the bases for a new cooperation between apidologists and climatologists has been laid.

Honey bees and plants react to climate changes differently.

While bee colonies tend to behave as complex endothermic superorganisms tuned by homeostatic mechanisms, plant metabolism is liable of strong changes depending on the environmental conditions.

In the new situation, gaps between the phenological stages of bees and of plants are likely to occur frequently, limiting the possibility that the colonies exploit nectar and pollen resources in critical periods for their development.

Less obvious but very serious as well, is the indirect effect of climate changes onto the colony through their influence on the life cycle of bee pathogens.

For instance, the ubiquitous haematophagous mite *Varroa destructor*, one of the major causes of colony collapse worldwide, needs a control strategy usually based on the combination between natural active ingredients, administered in different seasons. However, the external conditions may influence the global efficacy; in particular, positive temperature anomalies in winter and late-summer cools may reduce the acaricide release and/or unfavourably alter the host-parasite relationship.

Pollination is crucial for many wild and cultivated plants. Under this point of view, bees are of paramount importance to keep the agro-ecosystems balanced and productive. They are important factors of environmental, economical and social welfare at global level, and this is why large-scale negative effects can be expected by the current wave of poorly understood bee colony mortality.

Honey bee safeguard implies to attain a global understanding of the *honey bee colony wellbeing*. This concept is important for many reared species, but deep gaps in our understanding exist when remote organisms like insects are taken into consideration.

The superorganismic complexity of a colony makes the wellbeing condition even more complicate to assess when honey bees are taken into consideration.

Thus we need to combine different expertises and build a *wellbeing model* for the honey bee colony, including all the possibly involved internal and environmental factors, both of biotic and abiotic nature.

Additional Poster Presentations

- Daily summer fall of *Varroa destructor* calculated from short (1, 2, 3, and 4-week) sampling periods to be used as an indicator of autumn mite infestation of honeybee colonies (Bienkowska Malgorzata)
- Influence of season and degree of *Varroa destructor* contamination on the hygienic behaviour and ascospaerosis larvae sink rate of local Bulgarian honeybees (Petrov Plamen)

Demonstrations

- Demonstration of the “by eye” method for estimating bee colony populations (Seppo Korpela & Lauri Ruottinen)

List of participants

| | | | |
|----|------------|------------|-----------------------|
| 1 | Berg | Stefan | Germany |
| 2 | Bienkowska | Malgorzata | Poland |
| 3 | Bouga | Maria | Greece |
| 4 | Büchler | Ralph | Germany |
| 5 | Celle | Olivier | France |
| 6 | Costa | Cecilia | Italy |
| 7 | Dahle | Bjørn | Norway |
| 8 | Dyrba | Winfried | Germany |
| 9 | Garrido | Claudia | Germany |
| 10 | Hatjina | Fani | Greece |
| 11 | Heidinger | Ina | Germany |
| 12 | Ivanova | Evgeniya | Bulgaria |
| 13 | Kence | Meral | Turkey |
| 14 | Kezic | Nikola | Croatia |
| 15 | Korpela | Seppo | Finland |
| 16 | Kryger | Per | Denmark |
| 17 | Maori | Eyal | Israel |
| 18 | Meixner | Marina | Germany |
| 19 | Nanetti | Antonio | Italy |
| 20 | Nedić | Nebojša | Serbia |
| 21 | Panasiuk | Beata | Poland |
| 22 | Petrov | Plamen | Bulgaria |
| 23 | Ruottinen | Lauri | Finland |
| 24 | Uzunov | Aleksandar | Republic of Macedonia |
| 25 | Wilde | Jerzy | Poland |