Table of contents
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INTRODUCTION

Proceedings of the Second European Conference of Apidology EurBee bring texts of abstracts of oral and poster presentations. The texts of the participants are printed in the paper book form that it may be handy during the stay in Prague. Simultaneously you find the whole publication also on www.eurbee.org. From the security reasons the character @ is replaced by ©. The paper edition of Proceedings was compiled on the basis of e-mailed texts. For this reason we are aware of possible errors. We are convinced that you apologize involuntary shortcomings.

We ask you to read your texts and to send us kindly your corrections. We can correct the internet version of this Proceedings at any time.

We also would like to publish fulltext of your presentations in time. Fulltexts are to be published on www.eurbee.org. Would you be so kind to send us your texts and keep the deadline for the shipment of fulltexts December 31, 2006.

The Second European Conference EurBee has attracted more than 300 participants who present about 95 oral presentations and 170 posters. Under all scientific papers are signed 539 authors.

Prague becomes a meeting point of scientists and researchers not only from Europe but from the whole world. We believe that the most important benefit is just the personal meeting of many participants. We hope that this is the way how to fulfil the EurBee ambitions as stated by Norberto Milani in the introduction of the Proceedings book of the First European conference of Apidology EurBee in Udine in the year 2004: "bring together all the European researchers in different fields of Apidology and Apiculture, offering an opportunity to present recent advances achieved in Europe and promoting a multidisciplinary approach to open problems".

Dalibor Titěra
Local Organizing Committee
<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Session/Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunday</td>
<td>10</td>
<td>Registration, Welcome cocktail</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Registration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plenary session I - BIOLOGY</td>
</tr>
<tr>
<td>Monday</td>
<td>11</td>
<td>Plenary session II - GENETICS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lunch</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bee products</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Honey bee genetics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bee vision and learning</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Physiology and behaviour</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plenary session III - POLLINATION</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pollination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Macroparasites</td>
</tr>
<tr>
<td>Tuesday</td>
<td>12</td>
<td>Lunch</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diversity and conservation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BEESHOP - 6FP project conference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Management</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poster session</td>
</tr>
<tr>
<td>Wednesday</td>
<td>13</td>
<td>Excursion</td>
</tr>
<tr>
<td>Thursday</td>
<td>14</td>
<td>Plenary session IV - PATHOLOGY</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pathogens &amp; Diseases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Environmental Hazards</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lunch</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Honey bee viruses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Apis bees</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Round table discussions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gala Evening &amp; Traditional Dinner</td>
</tr>
<tr>
<td>Friday</td>
<td>15</td>
<td>Satellite meetings (IHC, BEESHOP Beekeepers forum)</td>
</tr>
</tbody>
</table>
**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plenary sessions</td>
<td>1</td>
</tr>
<tr>
<td>Symposia</td>
<td>7</td>
</tr>
<tr>
<td>Bee vision and learning</td>
<td>7</td>
</tr>
<tr>
<td>Physiology and Behaviour</td>
<td>11</td>
</tr>
<tr>
<td>Honey bee viruses</td>
<td>18</td>
</tr>
<tr>
<td>Pathogens and Diseases</td>
<td>27</td>
</tr>
<tr>
<td>Macroparasites</td>
<td>42</td>
</tr>
<tr>
<td>Honey bee genetics</td>
<td>51</td>
</tr>
<tr>
<td>Non-Apis Bees</td>
<td>61</td>
</tr>
<tr>
<td>Environmental Hazards to Honey Bees</td>
<td>79</td>
</tr>
<tr>
<td>Pollination</td>
<td>91</td>
</tr>
<tr>
<td>Bee products</td>
<td>115</td>
</tr>
<tr>
<td>Management</td>
<td>132</td>
</tr>
<tr>
<td>Beeshop</td>
<td>142</td>
</tr>
<tr>
<td>Authors Index</td>
<td>147</td>
</tr>
</tbody>
</table>
Plenary

REPRODUCTIVE BIOLOGY OF HONEYBEE (GENUS APIS) A UNIFORM PATTERN VARIED BY DIVERSE ADAPTATIONS

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The basic social structure of the honeybee colony seems to be congruent throughout the genus indicating that eusociality arose early in the common ancestors of the recent species. During the last decades, however, the exploration of African and mainly Asian honeybees resulted in a surprisingly high variability within and among the species. This detailed diversity of honeybees was primarily demonstrated by morphometric data (see Ruttner 1989) and more recently by thorough molecular and genetic analysis (see Oldroyd + Wongsiri 2005). A comparative synopsis of the reproductive biology is still missing and will be presented here. We focus on the following topics: production of queens and drones; sexual maturation; sex ratio; mating on the wings; sperm transfer; and sperm storage.

Queens
Brood cells for rearing queens are cone shaped and, in contrast to the “other” horizontal comb cells, are uniform with the longitude axis vertically orientated in all honeybee species. Queens are reared from fertilized eggs by feeding royal jelly to young larvae. The mechanism seems to be similar across species. Queenless colonies of Apis koschevnikovi accepted young female Apis cerana larvae and reared successfully Apis cerana queens.

Honeybee queens are regularly bigger than worker bees. These size differences, however, differ significantly among the species: in Apis dorsata queens are only slightly larger than worker bees and in Apis florea the queen’s weight is nearly two times more than the weight of a worker bee.

After emergence from the cell, surplus young queens fight and eliminate each other until monogyny is reached (1 queen per colony) and the origin of queen recognition signals seems to be located at the abdominal tergite glands. Experiments demonstrated that fights among queens occur across species borders. Not only queens of the closely related species Apis koschevnikovi and Apis cerana fight but also queens of Apis mellifera attacked young queens of Apis florea. The mechanism of recognition among young queens and the releaser of queen’s fighting behaviour seems to be similar within the genus Apis.

Drones
Brood cells for rearing drones are bigger than worker cells in all species. Mostly this is achieved by a bigger diameter and a domed sealing, although in Apis dorsata and Apis laboriosa the sealing is only slightly elevated. Further, drones are reared from unfertilised eggs. Queens can actively regulate the fertilisation of an egg; thus, she can regulate the number of drones per season.
Honeybee drones are regularly bigger than worker bees. These size differences, however, differ significantly among the species: drones in giant honey bees weigh only slightly more than worker bees, whereas in the dwarf species and in *A. mellifera* the drone’s weight is more than double the weight of workers.

Drones need about 12 days for sexual maturation. Only then are all sperm mobile and fit for transfer into the queen. The number of spermatozoa is dependent on the individual size of the drone. Environmental conditions like temperature during pupal development and care of newly emerged drones by workers may also affect sperm number.

**Sex Ratio**

The sex ratio in honeybees is male biased, the number of drones produced in a colony exceeds the number of queens by far. *A. mellifera* colonies produce 2 to 10 queens and 5 000 to 10 000 drones the relation is about 1 to 1 000. In *A. florea* 9.6 ± 1.3 queen cups and 568 ± 106 drone cells are built per season. If the drone cells are used only once per season, the relation is only about 1 to 60. In contrast to queens which take only 1 to 4 mating flights and almost always mate successfully, drones fly repeatedly until the age of up to 30 days and only few have a chance to mate. Thus the relation of queens to drones at the drone congregation area (DCA) is even more drone biased.

**Mating on the wings**

Mating never occurs within the colony. Drones and queens of all species perform mating flights and mating takes place outside the colony. Wind velocity, cloudiness and temperature are more critical for mating than for foraging, and mating flights occur only during favourable weather conditions. Mating flights take place at species-specific daily periods. Under allopatric conditions (only one *Apis* species), daily periods of mating occur around noon and are extended to the afternoon. Under sympatric conditions the mating periods are short and well separated among the different *Apis* species. Regularly under sympatric conditions, the sequence of mating periods correlates with the size of the drones, i.e. smaller species fly earlier than the larger ones.

The mating flight period seems to be independent of the colony rhythm. Drones of *Apis mellifera* which were kept in flight rooms under a shifted LD cycle were transferred to a colony outside and flew at first according to their shifted internal clock. Cross fostering experiments in Borneo confirmed the dominance of the individual rhythm. Drones and queens of *Apis koschevnikovi* and *Apis cerana* followed their species’ specific mating flight period regardless whether they were in alien or conspecific host colonies. Apparently, in all species queen’s mating flight period is relatively shorter and centred within drone’s flight period.

Congregation of flying drones above distinct locations that are used consistently over many years seems to a common pattern of most (if not all?) honeybee species. In *Apis mellifera*, drones assemble in the open air and their distribution seems to depend of physiographic factors. Although the DCAs of *Apis cerana* were found bordering the branches and canopies of trees, the *Apis cerana* drones flew under the open sky. *Apis koschevnikovi* drones, however, avoided congregating in the open and stayed under dense layer of branches. Also drones of *Apis dorsata* assembled under the canopy of trees. They chose large ,outstanding trees as a landmark and were flying at a height of 15 to 25m above the ground. The DCA of the dwarf bees (*Apis florea* and *Apis florea*) is still unknown and remains to be detected.
Also the sex attractants of the queen seem to be similar across species. A small black wooden dummy (pencil) contaminated by 1 microgram 9 - oxi decenoic acid attracted large numbers of drones when brought into the centre of a DCA for several different species. Further, when we offered a small hole in a sieve comparable to the queen’s sting chamber at the end of this, dummy drones copulated and got stuck by their everted endophalli. This was demonstrated in *Apis mellifera*, *Apis cerana* *Apis koschevnikovi* and *Apis dorsata*.

Sperm transfer

The monogamy of the male is typical for all species (drones die after copulation), whereas queens are highly polygamous. They mate, depending on species, with 12 or 50 or more drones on up to 4 mating flights. Drones get paralysed during copulation before transferring sperm and thus need mechanical support for keeping a strong connection between the flying queen and the paralysed drone until sperm transfer into the oviducts is completed. In most species this is accomplished mainly by the large endophallus, filled with mucus of the male accessory glands. In the dwarf species the hind legs have a special “thumb” to keep the connection of the copulating pair.

Sperm is transferred into the oviducts (cavity dwelling species) or into the ductus spermaticus (dwarf honey bees). When separating from the queen, drones of the cavynest species, and probably also of the giant honey bee species, leave a mating sign (secretions of 3 male accessory glands) which remains in the sting chamber. The next drone removes the mating sign of his predecessor and leaves his own.

In the cavity dwelling species, spermatozoa of many drones are present in the oviducts at the same time. Only small portions of them (3 to 8%) reach the spermatheca. In the dwarf honey bee species, this value is 40-06% after deposition of sperm into the ductus spermaticus. In spite of the high differences in sperm numbers and sperm transfer there is a uniform pattern in the paternity skew of the most frequent patrilines.

Sperm storage

Only 1-2 days after mating the queen starts egg laying. She uses 6 to 12 spermatozoa from the sperm storage in the spermatheca. We still do not know much about the biochemical mechanism of sperm storage. The spermatheca is surrounded by a dense tracheal net and a huge gland is connected to its lumen. The fluid of both contain sugars and many proteins. The protein concentration varies from 8.5 and 15.3 mg/mL in the spermathecal fluid, and from 5 to 8.5 mg/mL in the gland secretion. Until the age of 3 days the pattern of the gland secretion and spermathecal fluid was identical. In sexually mature queens (10 days or older) the gland secretion and spermathecal fluid each had one additional band at 79 kDa and at 29 kDa respectively. From the 29 kDa protein several peptide fragments were sequenced after digestion with LysC protease. Some of the sequences showed a distinct homology to the glycolytic enzyme triosephosphate isomerase (TPI), but the enzymatic activity was only 1/100 compared to TPI of hemolymph. The possible function of the protein is still under debate.

Generally, a loss or removal of a queen honeybee from a colony releases the construction of emergency queen cells and rearing of new queens. Further, queen loss has an effect on the development of the worker’s ovaries and sooner or later – if no new queen inhibits this effect - *Apis* workers start egg laying. The period between queen loss and worker egg laying varies between 6 and 30 days among tropical and temperate
subspecies of *Apis mellifera*. *Apis florea* (8 days) and *Apis cerana* (16 days) are positioned well in the range of *Apis mellifera*.

The reproductive biology of the genus *Apis* is shaped by the highly eusocial status of each species, and the uniform and strong influence of colony selection seems to be obvious. An individual selection, however, among queens and among drones surely proceeds as well. Looking at the fight between young virgin queens (see above) we can assume that individual selection would favour queens which hide from their sisters. The tergite glands, however, produce essential queen signals for workers and therefore queens cannot hide. Similarly, an individual “arms race” among drones would surely lead to a drone’s mating sign which effectively seals the queen’s bursa copulatrix and excludes competitors. The successful hiding queen and the sperm of the single drone, however, do not succeed in colony competition, which apparently favours queens with strong worker signals and populous colonies with a high genetic variance among its workers. We suggest that the uniform pattern of the reproductive biology of honeybees varied by diverse adaptations is a result of a limited individual selection and a final influence of colony level selection.

**THE EVOLUTION OF DIVISION OF LABOR AND FORAGING SPECIALIZATION**

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How does complex social behavior evolve? What are the developmental building blocks of division and labor and specialization, the hallmarks of insect societies? Recent behavioral, genetic, and genomic studies have revealed the developmental origins in the evolution of division of labor and specialization in foraging worker honey bees, the hallmarks of complex insect societies. Selective breeding for a single social trait, the amount of surplus pollen stored in the nest (pollen hoarding) revealed a phenotypic architecture of correlated traits at multiple levels of biological organization in facultatively-sterile female worker honey bees. Genetic mapping has demonstrated that the phenotypic architecture is a consequence of a genetic architecture rich in pleiotropy and epistasis possibly affecting a reproductive signaling pathway. Gene knockdown of a single hormone involved in reproductive signaling affects the entire phenotypic architecture and provides strong support for our hypothesis that division of labor and foraging specialization are derived from the reproductive cycle of solitary insects.
**BEE MOVEMENT AND POLLEN FLOW ACROSS THE LANDSCAPE**

*Juliet L Osborne*

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Bees are continually making choices about where and when to forage, and on which plant species. Individual bees make these foraging decisions based on their own experience, or that of others in the colony. The resulting spatial and temporal distribution of bees across the landscape is associated with the spatial and temporal arrangement of nectar and pollen sources. The movement patterns of individual bees, and consequent distribution of foragers affect and define the movement of pollen between entomophilous plants, and thus can drive gene flow between plant patches.

The growing sophistication of GIS techniques, the development of harmonic radar to track individual flying insects, and the ability to analyse genetic relatedness in both bees and plants, have together opened up the possibility of predicting plant pollen flow across the landscape from newly gathered knowledge of bee movement and distribution patterns. These predictions can then be tested with empirical evidence of pollen-mediated gene flow between plants.

I will describe recent studies investigating how bees explore the landscape to find forage, how they distribute themselves amongst patches or fields of plants, and I will discuss different approaches for translating this information into estimates of spatial patterns of plant gene flow, using examples from both crops and wild flowers.

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**PARALLEL DECLINES IN POLLINATORS AND INSECT-POLLINATED PLANTS IN NORTHWEST EUROPE**

*Koos Biesmeijer*

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Despite widespread concern about declines in pollination services, little is known about patterns of change in most pollinator assemblages. We have studied bee and hoverfly assemblages in Britain and the Netherlands, and find evidence of declines (pre vs. post 1980) in local bee diversity in both countries, whereas hoverflies show divergent trends. Depending on the group and country, declines are most frequent in habitat and flower specialists, in univoltine species and/or in non-migrants. In parallel with this, outcrossing plant species reliant on the declining pollinators have themselves declined relative to other plant species. Taken together, these findings strongly suggest a causal connection between local extinctions of functionally linked plant and pollinator species.
American foulbrood (AFB) is the most devastating bacterial brood disease not only able to kill individual larvae but also resulting in colony collapse. The causative agent of AFB is a spore-forming, gram-positive bacterium, recently reclassified as *Paenibacillus larvae* (*P. larvae*). We could show that the species *P. larvae* consists of at least four ERIC-genotypes which differ morphologically, biochemically and, most importantly, in respect to virulence. Exposure bioassays revealed genotypic differences in the time it took the pathogen to kill the infected individuals. Based on these results we hypothesized that the observed differences in individual virulence will have an impact on the hygienic removal of diseased larvae and, therefore, disease progression in the colony. Experimental infection of mini colonies confirmed this hypothesis. The impact of these findings for disease progression both, within and between colonies and for the evaluation of the disease status of a colony will be discussed.
Symposia

Bee vision and learning

Symposium organized by Martin Giurfa and Lars Chittka

SEARCHING FOR FLOWERS - WHEN DO HONEYBEES GET CONFUSED?

Johannes Spaethe¹ and Lars Chittka²

¹ Department of Evolutionary Biology, University of Vienna, Austria
² School of Biological Sciences, Queen Mary, University of London, GB

While flying over a meadow and searching for a specific flower species a bee may detect several different flower types per second and thus the task of choosing the right flower and ignoring the others is not trivial. To investigate how the simultaneous occurrence of several flower types together with the sought-after flower species within the visual field of a bee may limit search accuracy we applied the concept of visual search from human psychology to the honeybee. Bees were trained to choose a colored disc (target) among a varying number of differently colored discs (distractors). We measured accuracy and decision time as a function of distractor number and color. We found that for all color combinations, decision time increased and accuracy decreased with increasing distractor number, whereas performance increased when more targets were present. These findings are characteristic for a serial search in humans, when stimuli are examined sequentially. Additionally, decision time and number of errors were found to be significantly higher when bees had to choose a blue target among yellow distractors compared to the inverse color combination, a phenomenon know as search asymmetry in humans.

CAN HONEYBEES LEARN BY EXAMPLE?

Lars Chittka, Ellouise Leadbeater, Nigel Raine

School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK

Social learning in insects has received much less attention in insects than in vertebrates, but the literature contains several intriguing examples. Darwin suggested that honeybees can copy from bumblebees how to rob nectar from long-spurred flowers, by observatory learning. Indeed, new research indicates that there might be cases of social learning in flower-visiting bees. Recent publications even claim that teaching might occur
in some social insects. Could the honeybee dance be a case that contains both observatory learning by recruits and teaching by dancers? We discuss the evidence and possible experiments, including the feedback from recruits to dancers, that would be necessary to answer these questions.

**Categorization of Visual Stimuli in the Honeybee Apis Mellifera**

*Julie Benard, Geoffrey Portelli, Martin Giurfa*

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Categorization refers to the classification of perceptual input into defined functional groups. We present and discuss evidence suggesting that stimulus categorization can also be found in an invertebrate, the honeybee Apis mellifera, thus underlining the generality across species of this cognitive process. Honeybees show positive transfer of appropriate responding from a trained to a novel set of visual stimuli. Such a transfer was demonstrated for specific isolated features such as symmetry or orientation, but also for topographic layouts of features. Such assemblies may involve different orientations of a single element (e.g., a bar), or even different elements building structured or unstructured face-like stimuli. Moreover, bees could learn very well to categorize short- versus long-wavelength stimuli (as they discriminate between and generalized within categories) and they generalized successfully their choice to new colors belonging to these categories. Though in most cases specific experimental controls such as stimulus balance and discriminability are still required, it seems appropriate to characterize the performance of honeybees as reflecting categorization. Further experiments should address the issue of which categorization model accounts better for the visual performances of honeybees.

**Molecular Basis Underlying the Mushroom Body Function of the Honeybee Brain**

*Hideaki Takeuchi*

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The honeybee is a social insect and its colony consists of a queen, workers and drones that have different roles. In addition, various complex social behaviors, such as communications and divisions of labor, are performed by colony members to maintain colony activities. Mushroom body (MB) is one of insect brain structures, which is important for learning, memory and sensory integration. Honeybee MB function is believed to be closely associated with honeybee social behavior for the following reasons: 1) The MBs of the aculeate Hymenoptera, including the honeybee, are more prominent compared with those of other insects. 2) Honeybee MBs have a high degree of structural plasticity and the volume of the neuropil varies according to the division of labor. To clarify molecular basis underlying honeybee MBs function, we identified over 20 genes expressed selectively in the MBs of the honeybee brain using combination of
differential display and cDNA microarray method. In this talk, I will present the expression pattern of these MB-selective genes and discuss possible function of these genes in comparison with those of their orthologues in non-social insects/invertabrare. We also study about visual learning and chromatic adaptation in the harnessed honeybee and will examine involvement of these genes in the honeybee visual behaviors.


ASSOCIATIVE VISUAL LEARNING, COLOR DISCRIMINATION, AND CHROMATIC ADAPTATION IN THE HARNESSED HONEYBEE

Sayaka Hori¹, Hideaki Takeuchi¹, Kentaro Arikawa², Michiyo Kinoshita², Naoko Ichikawa³, Masami Sasaki³, Takeo Kubo¹

¹;Department of Biological Sciences, Graduate School of Science, The University of Tokyo 2; Laboratory of Neuroethology, The Graduate University for Advanced Studies, 3; Faculty of Agriculture, Tamagawa University, E-mail: holis@biol.s.u-tokyo.ac.jp

To analyze visual cognitive capacity in the honeybee, we studied associative visual learning in harnessed honeybees trained with monochromatic lights associated with a sucrose solution, to elicit the proboscis extension reflex (PER). We clarified five properties of visual learning. 1, Antennae deprivation significantly increased the learning rate, suggesting that sensory input from the antennae interferes with the light-PER associative learning in this conditioning paradigm. 2, Shading the compound eyes with silver paste significantly decreased the learning rate, while shading the ocelli did not. These results indicate that the visual information received by the compound eyes was crucial in this conditioning paradigm. 3, There was no significant difference in the visual learning between nurse bees, guard bees, and forager bees. These results indicated that these workers have almost equivalent ability for light-PER associative learning. 4, Bees conditioned with 540nm light stimulus exhibited light-induced PER with 618nm, but not with 439nm light stimulus. These results indicate that the 540nm light-conditioned bees generalized between 618nm and 540nm light stimuli, whereas they did not generalize between 540nm and 439nm. 5, Bees conditioned with 540nm light stimulus exhibited PER immediately after the 439nm light was turned off, suggesting that the bees reacted to an afterimage induced by prior adaptation to the 439nm light, which might be similar to 540nm light.

HONEYBEE WORKERS EXCEL IN DISTINCTION AND LEARNING OF COLORS, SHAPES AND PATTERNS

Sayaka Hori

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To analyze visual cognitive capacity in the honeybee, we studied associative visual learning in harnessed honeybees trained with monochromatic lights associated with a reward of sucrose solution delivered to the proboscis, to elicit the proboscis extension
reflex (PER). We clarified five properties of visual learning under these conditions. First, antennae deprivation significantly increased the learning rate. These results suggested that sensory input from the antennae (olfactory and/or mechanical stimulation) interferes with the light-PER associative learning in this conditioning paradigm. Second, shading the compound eyes with silver paste significantly decreased the learning rate, while shading the ocelli did not. These results indicate that the visual information received by the compound eyes was crucial in this conditioning paradigm. Third, there was no significant difference in the visual learning between nurse bees, guard bees, and forager bees. These results indicated that these workers have almost equivalent ability for light-PER associative learning, independent of their roles in the colony. Forth, bees conditioned with 540nm light stimulus exhibited light-induced PER with 618nm, but not with 439nm light stimulus. These results indicate that the 540nm light-conditioned bees generalized between 618nm and 540nm light stimuli, whereas they did not generalize between 540nm and 439nm. Finally, bees conditioned with 540nm light stimulus exhibited PER immediately after the 439nm light was turned off, suggesting that the bees reacted to an afterimage induced by prior adaptation to the 439nm light, which might be similar to 540nm light. A combination of behavioral and neurobiological methods will contribute to clarify the neural circuit and molecular mechanisms underlying visual processing in honeybees.

BEE ORIENTATION UNDER NEW ENERGY SAVING GREENHOUSE CLADDINGS

Tjeerd Blacquière, Bram Cornelissen, Jeroen Donders

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New greenhouse cladding materials are developed with the aim to reduce the loss of heat from the greenhouse, and simultaneously to maximize the transmission of light for plant growth (wavelengths 400-700 nm). The new claddings are made of polymers, with transmission characteristics differing from those of glass, and double layered, while glass is only one layer. How do honeybees behave and forage under these conditions? The signals and information they normally use are absent or at least altered: reduced sight on the position of the sun, loss of polarization of light that has passed at least four times a gas-solid boundary and loss of ultra-violet radiation. In addition a greenhouse is in general as such a difficult surrounding for bees because of the lack of landscape variation, the use of screens that turn normal light distribution upside down and behind which bees easily get confused and lost, and vents with or without insect gauze.

On a nursery with compartments with different claddings the orientation behaviour of worker bees of newly introduced colonies in small hives (MiniBeuten) was registered. Counts were made of the number of bees that went out for an orientation flight and of the numbers that returned to the hive. The claddings were glass, polymethylmethacrylate (PMMA) and polycarbonate (PC).

Honey bees leaving the hive behaved normal under glass and PMMA, by flying in circles increasing in width around the hive, but divergent under PC, where they almost immediately went up and with a few loops ended in the top of the greenhouse on the insect gauze of the vents. Beneath glass and PMMC many bees returned to the hives, beneath PC hardly any bee returned.

Bumblebees experienced similar problems in the PC greenhouse.
Differences between foraging groups in a learning task but no correlation in performance between tasks

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Due to multiple matings by the queen, a honey bee colony consists of foragers from several patrilines. These patrilines are known to differ in their tendency to collect pollen or nectar, and in various learning tasks. We tested returning foragers in a simple learning task by conditioning of the proboscis-extension response (PER). Subjects received three acquisition trials in which an odor was associated with a sucrose reward. Bees that had foraged for pollen and water learned better than those that foraged for nectar or for both nectar and pollen. Subjects were then tested in a PER assay modified for simultaneous choice between two odors, for their preference between an odor associated with a constant reward and another associated with a variable reward. Bees were risk averse in that they preferred the constant alternative, but levels of risk aversion did not differ between the various foraging groups. Finally, subjects were tested for latent inhibition. They experienced 30 unrewarded trials of exposure to an odor, followed by six acquisition trials with that odor. Learning of the inhibiting odor during the inhibition phase was manifest by retarded learning during the acquisition phase for the latent inhibition group relative to a control group, but there were no differences between the foraging groups. Our results suggest that different genes govern foraging specialization and performance in various learning tasks.

Do honeybees, Apis mellifera scutellata, regulate humidity in their nest?

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Honeybee colonies are highly efficient at regulating the biophysical parameters of their hive. Constant temperature is crucial for the normal growth of the immature stages, and temperature regulation has been studied extensively. Humidity is also essential for brood development as well as for osmotic balance of the adults, but in spite of its important role in nest homeostasis, little is known about whether and how this parameter is regulated by the honeybees. Using miniaturised technology, we measured humidity at
different positions inside beehives, and have begun investigating whether this parameter is actively regulated by the workers. We obtained evidence that the honeybee workers influence hive humidity, but do not regulate it precisely. Optimal humidity differs for brood area and nectar stores, resulting in constraints on potential regulation mechanisms in a shared atmosphere. The availability of external water may further impair regulation and the maintenance of optimal humidity levels may be disrupted as a result of trade-offs between temperature regulation and respiratory gas exchanges. Therefore, we argue that workers can only adjust humidity within sub-optimal limits.

HOW DO BABY BEES FIND A TEMPERATURE OPTIMUM

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The behaviour of a honeybee colony depends on many decisions of single honeybees. To understand the behaviour of such a superorganism, the understanding of the individual behaviour is essential. Especially understanding the ways of individual decision making is necessary to explain the ultimate colony behaviour, by which the colony reacts adequately to changes inside and outside of the colony. Freshly emerged honeybees need contact to certain stimuli within the colony (like brood-pheromones) to develop into fully-fledged members of the colony. The first task in a honeybees¡ life is the “cleaning” task, which is performed mostly inside the broodnest, preparing cells for the egg-laying queen. Due to this, the presence of young bees in the broodnest area is advantageous for the colony. The self-organised navigation principles of one-day old bees were investigated in a laboratory experiment: We built a comb sized arena with an artificial scalable heat-gradient, with a maximum of about 36°C. We observed the optimum-seeking behaviour and the social interactions of young bees in different shapes of gradients. We found, that bees show different kinds of uphill-going behaviour and different clustering behaviour in dependence of the steepness of the temperature gradient they were exposed to.

AGE SPECIFICITY IN EXPRESSION OF FAT BODY PROTEINS IN HONEYBEE (APIS MELLIFERA L.) DURING ONTOGENESIS

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During the last years the electrophoretic investigations of a number of proteins and enzymes play a significant role in the solution of population-genetic and ontogenetic problems but very few electrophoretic analyses have been published yet to describe the age specificity of protein expression in the fat body of the male and female individuals of A. mellifera during their ontogenesis. The aim of this investigation was to elucidate and analyze some peculiarities in the age specificity of the protein expression in the fat body of A. mellifera during the development of the workers and the drones.
Using 7.5% PAGE, 184 individual samples of fat body extract were investigated. The workers were studied after laying the eggs in their larval stage (5th day), pre pupal (10th and 11th days) and pupal (14th to 20th days) stages and before flying off the imago (21st day). The drones were studied after laying the eggs in their larval stage (5th and 10th days), pre pupal (14th days) and pupal (18th to 22nd days) stages and before flying off the imago (24th day).

A total of 18 protein fractions (SP-1 to SP-18) were expressed in the fat body of male and female individuals during their development. There were found specific proteins for different ages of individuals. Some sex-differences in studied fat body proteins were noticed. The age and stage specific dynamic in the expression of the established proteins of the fat body spectrum was analyzed and commented.

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RNA INTERFERENCE AND OLFACTORY MEMORY IN THE HONEYBEE

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Acetylcholine is involved in olfactory learning in the honeybee through the activation of the nicotinic acetylcholine receptors (nAChRs). Classically, the nAChRs are formed of an assembly of different α and β subunits but in insects, we do not know the molecular composition and the pharmacological properties of these receptors. Nine α and 2 β subunits have been identified recently from the honeybee genome, each of these subunits could potentially combine to the others to form functional nAChRs. For a better understanding of the role of the nAChRs in insect memory, we induced silencing of the α3 subunit using the technique of RNA interference (RNAi). RNAi was injected into the honeybee brain through the median ocellus and the protein expression was evaluated at different times after injection using Western blot analysis.

A significant decrease of the α3 protein was observed 6 hours after injection. The effect of the protein suppression was evaluated on the olfactory conditioning of the proboscis extension reflex, using 3 trial olfactory learning. RNAi injection was performed 6 h before the learning session or before the memory test. Saline and RNAi against GFP were used as control. Pre-training injection had no effect on learning performance and induced a response generalization to unknown odorant 24 h after training. RNAi injected 6 h before the retrieval test unexpectedly increased the memory performance. A discriminative olfactory learning is currently under investigation to test generalization phenomenon in honeybees lacking the α3 subunit.
PROTEIN SUPPLEMENTATION IN HONEYBEE COLONIES – WHERE DOES THE PROTEIN GO?

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Feeding of protein containing food to honeybee colonies is occasionally recommended in spring to compensate for a lack of pollen. These feeding should supply the nurses with additional protein and thus increase breeding activity after the end of winter.

We applied a mixture of yeast and icing sugar containing non toxic stains in different areas of observation hives and analysed the pattern of consumption by behavioural observations and photospectrometric analysis. We found that this food offered was distributed throughout the colony either by direct consumption or by a trophallactic pathway. The stains were found in larval food for various ages as well as in honey. Thus the protein in the artificial food did not go just to the nurses but was fed in no processed form to the larvae and to other bees. The fact that it could be found in honey also indicates a contamination of this bee product with protein.

The meaningfulness of protein supplementation for honeybees is discussed controversy in literature. We did not test the effect of the provided protein but could show that the protein is rather uniformly distributed than selectively utilized by a special cohort of bees.

FIPRONIL EFFECT ON SIDE-SPECIFIC ANTENNAL TACTILE LEARNING IN THE HONEYBEE

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Individual restrained honeybees were submitted to side-specific tactile learning of the proboscis extension response. In this task, a tactile stimulus delivered to one antenna is associated to a reward of sucrose solution delivered to the proboscis. Two tactile stimuli, A and B, were used as conditioned stimuli in a 6 trial learning paradigm. In the first learning situation, one antenna was stimulated with A and the honeybee was reinforced with sucrose and the other antenna was not stimulated (A+/0). In the second situation, A presented to one side was reinforced and B was presented to the other side non-reinforced (A+/B-). In the third situation, A was learned on one side and B was learned on the other side (A+/B+). During retrieval tests, both stimuli were randomly presented to each antenna 3 h and 24 h after training. Fifteen min. before learning, the animals received a thoracic application (1 µl) of fipronil (0.1 ng) or of acetone solvent (control group). In (A+/0) and (A+/B+) training situations, control animals responded to A+ on both sides, showing a transfer of information between sides. No generalization of the conditioned response to B- was observed. When honeybees specifically learned two different stimuli on each side (A+/B+), they showed side-specific response patterns, excluding transfer and generalization phenomena. Fipronil-treated honeybees had lower performance than control bees in the (A+/0) and (A+/B+) trainings and did not discriminate between A+ and B- in the (A+/B-) training. In the retrieval tests, these
honeybees responded to both stimuli on both sides with a memory loss at 24 h. We conclude that a sublethal dose of 0.1 ng/bee deteriorates side-specific learning abilities of honeybees.

**DOMINANCE HIERARCHIES IN EXPERIMENTAL GROUPS OF HONEYBEE WORKERS**

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Under queenless conditions worker bees are able to activate their ovaries (Ruttner and Hesse 1981), but only few start oviposition and secretion of a queen-like pheromonal bouquet in their mandibular and Dufour’s glands after a short period of extreme intracolonial selection. Such ‘pseudoqueens’ (Velthuis et al. 1990) can suppress ovary activation and the production of queen-like pheromone signal in other workers (Moritz et al. 2000). In this set of experiments the effect of group size on dominance patterns in queenless groups was tested in petri dishes using freshly emerged and individually labelled bees ranging from 2 to 10 workers. Due to the queenless conditions workers were initially unexposed to 9-ODA and could gradually increase their level of pheromonal production over the experimental time. Recently Moritz et al. (2004) analyzed the dynamics of pheromone production in paired workers. They found that queenless workers engage in a pheromonal contest and compete for reproductive dominance, finally resulting in a stable hierarchy of dominant and subordinate workers. With increasing group size, it is difficult to predict whether workers participate in clear-cut reproductive hierarchies dominated by a single worker or whether they will exhibit a continuous range of pheromonal levels with several dominant workers. The aim of the experiment was to quantify social hierarchies in different group sizes, using the highly sensitive GC-analysis of the mandibular gland pheromones (queen-worker substance ratio).

**DEPENDENCE OF THE MORPHOLOGICAL CHARACTERISTICS OF THE BEE VENOM GLAND OF THE HONEY BEE ON THE PARAMETERS OF THE THIRD TERGIT**

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This investigation is one of a set of studies carried out on the morphological characteristics of the bee venom gland of the honey bees in Bulgaria.

The aim of the investigation is the establishment of the correlative dependence of the morphological characteristics of the bee venom gland on the parameters of the third tergit of Apis mellifera Sp.L. honey bee in Bulgaria.

The investigation has been carried out at the experimental base of Apiculture at the Agricultural Faculty at the Trakia University in Stara Zagora. 87 marked honey bees from a honey bee colony at the age of 30-40 days have been used. The sting, together with the reservoir and the bee venom gland of each mortified bee has been measured. The length of the trunk of the bee venom gland, the length and the width of the reservoir
have been measured by a microscope. The volume is calculated by the following formula: $V=(0.5.a)^2 \pi.b/3$, where $\pi=3.14$; $b$ and $a$ are the length and the width of the reservoir. The third tergit has been separated and measured following the method of Alpatov.

In this investigation the length, the width and the volume of the reservoir have been studied together with the length, the width and the distance between the tentacles as included in this investigation parameters of the third tergit.

The statistical calculation of the data includes: the receiving of the basic statistical numbers of all mentioned measurements, the calculation of the coefficients of the correlation between them, the receiving of the regressive equations for all measurements depending on the parameters of the third tergit. Two models for calculation of the regressive equations have been used in the investigation:

$$Y_j=\mu+\Sigma a_i x_i, \ i=1,2,3 \ (1)$$
$$Y_j=\Sigma a_i x_i, \ i=1,2,3 \ (2)$$

where $\mu$ is the average number for the studied population, $Y_j$ is one of the measurements of the bee venom gland, the variable quantities $x_i$ are the width, the length and the distance between the tentacles as parameters of the third tergit, together with coefficients in the equation $a_i$.

Statistica for Windows has been used for the calculations.

The established coefficients of the correlation between the used measurements and the linear regressive equations are statistically reliable. The established correlations and equations could be used for the selection of honey bee colonies by the signs of the honey bee venom gland. They could also be used for making a forecast of the measurements of a honey bee colony and for a forecast of the yield of honey bee venom.

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**A TECHNIQUE FOR MEASURING TEMPERATURE DISTRIBUTIONS IN THE COMB OF HONEYBEES (APIS MELLIFERA)**

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In order to save energy, honey bees (Apis mellifera) are able to form clusters, when ambient temperatures fall below a threshold of about 18°C. Those winter clusters consist of an outer mantle insulating a warmer core region and are maintained by the consumption of honey stores for metabolic heat production. To analyse temporal and spatial temperature distribution in honey bee combs, we constructed a precise thermo-device, providing us constantly with temperature data of 768 cells. These data are coupled with a simultaneous video record of the comb allowing for the combination of worker allocation on and temperature distribution in the comb. Here we test different group sizes of clustering bees on the heat transfer to the comb under cool temperature conditions. Efficient heat accumulation requires a least number of individuals. We investigated the relationship between number and distribution of the bees and steepness of the temperature gradient on the test-comb in dependence of differing ambient temperatures.
CANDIDATE GENES INVOLVED IN THE HONEYBEE SOCIAL BEHAVIORS

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The honeybee is a social insect and its colony consists of a queen, workers and drones that have different roles. In addition, various complex social behaviors, such as communications and divisions of labor, are performed by colony members to maintain colony activities. Mushroom body (MB) is one of insect brain structures, which is important for learning, memory and sensory integration. Honeybee MB function is believed to be closely associated with honeybee social behaviors for the following reasons: 1) The MBs of the aculeate Hymenoptera, including the honeybee, are more prominent compared with those of other insects. 2) Honeybee MBs have a high degree of structural plasticity and the volume of the neuropil varies according to the division of labor. To clarify molecular basis underlying honeybee MBs function, we identified over 20 genes expressed selectively in the MBs of the honeybee brain using combination of differential display and cDNA microarray method. In this talk, I will present the expression pattern of these MB-selective genes and discuss possible function of these genes in comparison with those of their orthologues in non-social insects/invertabrate. We also study about visual learning and chromatic adaptation in the harnessed honeybee and propose our strategy to examine involvement of these genes in the honeybee visual behaviors.

Reference
Honey bee viruses

Symposium organized by Joachim de Miranda

IMPACT OF VIRUS INFECTIONS IN HONEY-BEES

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Field observations on the incidence of honey bee virus infections suggest that honey bee individuals as well as colonies may suffer severely from the infections. Furthermore, it seems likely from field reports that the development of overt infections is context dependent. In other words, honey bees may carry infections but suffer little or no consequences, but given other conditions the infection may become overt and the impact fatal at colony level, even from rather limited effects at the individual bee level. Nevertheless, it is necessary to unravel the causal relationship between colony deaths and virus infections, since available field data are not conclusive.

For many bee viruses, pathogenicity is still poorly understood. However, field observations show that several virus infections are associated with honey bees symptoms and significant mortality. Viruses are among the simplest parasitic forms, they are widely spread and always ready to adapt themselves to new conditions – included those created by beekeepers. Moreover, the recent introduction of Varroa destructor demonstrated that conditions and routes of transmission may be dramatically changed. Problems with honey bee viruses are real, they require more appropriate studies to be assessed and controlled.

COLONY MORTALITY AND BEE VIRUSES

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Sensitive molecular detection methods showed the presence of bee viruses in apparently healthy colonies, sometimes even with high virus titres (Tentcheva et al. 2004). The aim of our study was to compare prevalence of viruses in healthy and diseased colonies.

At the end of the winter, beekeepers announced apiaries with abnormally high colony mortality. Bee samples were taken from dead, weak or apparently healthy colonies in these apiaries and from healthy colonies in control apiaries. Pools of 50 bees were analysed for the presence of Acute Bee Paralysis Virus (ABPV), Deformed Wing Virus (DWV), Chronic Bee Paralysis Virus (CBPV) and Kashmir Bee Virus (KBV). Positive samples were then analysed with a quantitative RT-PCR method.

Results showed that neither CBPV nor KBV were detected. In control apiaries, ABPV was never found and low DWV titers were found in only 25% of the colonies
tested. In diseased apiaries, all colonies were DWV positive with significantly higher virus loads. Moreover, about 70% of colonies were also ABPV positive.

The differences observed between healthy and diseased colonies indicate that ABPV and DWV could play a role in colony mortality during the winter. A reduced bee life span known to be associated with virus infections could explain the colony mortality. However, the factors inducing high virus titres, like mite infestation, are to be further studied.

**Susceptibility on virus infections of different bee strains – a field study**

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Within the scope of the German varroosis tolerance breeding project we compared five bee lines concerning the virus infections. The colonies were kept during two years without treatment against varroosis. Bee samples were taken from the colonies throughout this period. We analyzed four viruses (ABPV, KBV, SBV and DWV) by means of PCR technique. Additionally, the viral load of ABPV and DWV was analyzed by means of RT-PCR technique.

We found differences in the patterns of virus infections of the different bee lines. The correlation to colony development, overwintering performances and Varroa infestation level is discussed.

**A real-time PCR based survey on acute bee paralysis virus in German bee colonies**

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The acute bee paralysis virus (ABPV) is pathogenic for honey bees (Apis mellifera). ABPV is a small, single-stranded RNA virus with positive polarity. Recently ABPV was classified as a tentative species of the genus Cripavirus (Fam. Dicistroviridae). It was suggested that the virus is firmly associated with the bee-parasitic mite Varroa destructor. High amounts of ABPV were found in bees from colonies which died of varroosis. The parasite is believed to represent a vector for ABPV and to be important for fatal ABPV outbreaks.

Here we report a field survey of 11 German apiaries which were followed up for two years. In autumn 2004 and 2005 worker bees were skimmed off from combs of ten bee colonies per apiary. In march the survivorship from the previous winter of each colony was recorded. The mite infestation in autumn and the colony strength before and after winter was registered. Out of the group of the surviving hives three colonies per apiary were randomly selected for analysis of their ABPV-load. All bee samples which
derived from collapsed colonies and heavily damaged bee yards were assessed for ABPV. For this purpose a SYBR-green real-time PCR assay was developed.

Much variation of the ABPV burden occurred during the two years between the colonies and between the yards. The ABPV burden was linked to the number of mites. The presumption of a linkage between the ABPV load and the colony strength after winter was investigated. Interestingly, higher ABPV loads were followed by colony deaths. Our data suggest that the amount of ABPV in autumn can be correlated with poor outcome in winter.

**PREVALENCE AND PHYLOGENY OF KAKUGO VIRUS IDENTIFIED FROM AGGRESSIVE WORKER HONEYBEES (APIS MELLIFERA L.) UNDER VARIOUS COLONY CONDITIONS**

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We previously identified a novel insect picorna-like virus, termed Kakugo virus (KV), from the brains of aggressive worker honeybees that had counterattacked a giant hornet. To examine the relation between KV infection and aggressive worker behavior, we surveyed the prevalence of KV in various worker populations by quantifying KV genomic RNA. KV was detected specifically from aggressive workers in some colonies, while it was detected also from other worker populations in other colonies where the amount of KV detected in the workers was relatively high. This finding suggests that in the primary infection phase KV infection is attacker specific, whereas in the late phase KV is infectious to various worker populations. To investigate whether KV strains detected were identical, phylogenetic analysis was performed. There was a less than 2% difference in the RNA-dependent RNA polymerase (RdRp) sequences between KV strains from aggressive workers and those from other worker populations, and these strains had approximately 6% and 15% sequence differences in the RdRp region from deformed wing virus and Varroa destructor virus-1, respectively. These results suggest that all of the viruses we detected were virtually the same KV and KV represents a closely related but distinct viral strain from the other two viruses. We also found some of the KV-infected colonies were infested with Varroa mites and sequences of the KV strains detected from the mites were the same as those detected from the workers of the same colonies, suggesting that the mites mediate KV prevalence in the honeybee colonies.
Honey bee viruses

THE PITFALLS OF DIAGNOSIS INTERPRETATION IN HONEY BEE PATHOLOGY. THE CASE OF DEFORMED WING VIRUS (DWV)

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Among the fifteen distinct viruses identified in Apis mellifera L., the deformed wing virus (DWV) is one of the most prevalent in honey bee colonies. The high prevalence of DWV is likely correlated to its ability to be transmitted by the mite Varroa destructor. The PCR amplification of DWV negative RNA strands in mites and the tremendous amounts of DWV genome copies recorded from mites argue for the replication of DWV in varroa and in bee as well. Besides, there is strong evidence that DWV is also transmitted between individuals of the colony, either horizontally by food exchange or vertically through the queen’s eggs.

We attempted to measured DWV RNA loads in 360 seemingly healthy honey bee colonies from pools of 100 bees using quantitative PCR. Our data showed that honey bee colonies can tolerate very high loads of viruses without displaying external clinical signs. We further identified DWV RNA in several bee organs using the in situ hybridization technique and showed that queen and drone fertility could be impaired by such infection. In queen, the fat body cells were particularly infected while in drone, the whole reproductive tract was clearly stained with the DWV probe. Moreover, in crippled winged individuals from where very high DWV RNA genome copies were recorded, the digestive tract was heavily stained with the DWV probe, indicating a probable negative effect of DWV infection on the digestive function of the bee.

Our data strongly support that DWV produces pathogenic effects in severely infected individuals from the colony. However, when considering the colony level, these deleterious effects observed on individual bees might not always have an impact on the colony fitness.

INFECTIOUS CHRONIC BEE PARALYSIS VIRUS (CBPV) EXCRETION IN HONEY BEE (APIS MELLIFERA L.) FECES: A WAY OF SPREAD

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Knowledge of the spreading mechanism of honey bee pathogens in the hive is crucial to our understanding of the dynamics of bee disease. The aim of our study was to assess the presence of infectious CBPV in bee excreta and to evaluate its possible role as an indirect route of infection. Batches of or individual infected bees were produced by experimental inoculation with purified virus or collected from colonies with paralysed bees. CBPV in bees (bee heads) or faeces (fresh or absorbed onto paper) was detected by reverse transcription-polymerase chain reaction (RT-PCR). CBPV RNA was detected in faeces of naturally and experimentally infected bees and on the sheets of paper that were used for covering the floor of units that had contained artificially CBPV infected bees or for covering the floor of naturally infected colony. Additionally CBPV infectivity was assessed by intra-thoracic inoculation of naive bees with faeces extracts or by placing naive bees in cages previously occupied by contaminated individuals. Both procedures
entailed overt disease in naive bees. To our knowledge this is the first experimental confirmation that infectious CBPV particles excreted in faeces of infected bees can infect naive bees by mere contact and provoke overt disease.

**THE BRAVE PROJECT**

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Built on EurBee initiatives, the European BRAVE project (Bee Research And Virology in Europe) (duration: the year 2005) was aimed at knowledge transfer between experts in insect virology and scientists involved in research on bees and related species. Furthermore, the project aim was to synthesise the current knowledge required for protecting the honey bee and related pollinator insects from virus diseases and to propose a framework for future research programmes in this field.

More than 60 world experts exchanged during a meeting in Sophia-Antipolis (France) in April 2005. They explored the taxonomy of bee viruses, diagnostic techniques and their appropriateness. Genetics, physiology and behaviour of honey bees were studied in relation to their resistance to virus infections. The association of virus infections with parasites such as Varroa destructor or Nosema apis, and the possible depression of the honey bee immune response by exposure to sub-lethal doses of pesticides were also raised.

The evolutionary epidemiology of virus diseases, current information on the incidence, distribution and impact of honey bee viruses, the management of bee diseases including the regulatory mechanisms governing the world exchanges were all covered. The proceedings of this meeting have been published [ww.entom.slu.se/BRAVE/publications.htm](http://ww.entom.slu.se/BRAVE/publications.htm). They include the scientific texts and the recommendations elaborated by the working groups and approved in plenary sessions.

A workshop meeting took place later at which several experts have prepared a synthesis of current knowledge in these areas. This task will be published in the form of a book entitled "Virology and the Honey Bee" whose publication is expected at the end of this year.

The European BRAVE project will have produced tangible results: the proceedings of the BRAVE plenary scientific meeting and the comprehensive book "Virology and the Honey Bee".

**BLACK QUEEN CELL VIRUS IN COMMERCIALLY REARED QUEEN BEES**

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Black queen cell virus (BQCV) has been found to be a cause of death of queen prepupae and pupae. In adult bees it almost invariably multiplies only in those individuals that are also infected with Nosema apis. Recent studies conducted in
Maryland (USA) revealed the presence of the virus in 100% of queen bees which were collected for examination from randomly chosen colonies. From our previous investigations of queen cells, we draw the conclusion that queen rearing apiaries can differ considerably as to the level of BQCV infection of reared queen bees.

In this work we examined for presence of BQCV unmated queens sold to bee keepers by four queen rearing apiaries. The queens were reared in May and August. BQCV was found only in the queens reared in one apiary but both in May and August. These queens were free from Nosema apis spores so we concluded that they had become infected with the virus through ingestion of contaminated food during the larval rather than the mature stage.

STUDY OF CHRONIC BEE PARALYSIS VIRUS (CBPV) REPARTITION IN HONEY BEE (APIS MELLIFERA) COLONIES

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Knowledge of the virus distribution in bee colonies and of the kinetics of virus load are crucial to our understanding of Chronic paralysis infection dynamic. This study investigated CBPV infection in several bee colonies either symptomatic or asymptomatic of the same apiary. The CBPV genomic loads of a variety of brood samples (with eggs, larvae or pupae), home, guard, forager bees, and symptomatic and dead bees collected from the flight board were quantified by a real-time TaqMan RT-PCR assay. Results obtained from symptomatic or dead bees from colonies with Chronic paralysis confirm the correlation between high CBPV genomic load and pathology expression. Surprisingly, highest CBPV genomic loads were not observed in the group of the oldest bees (the foragers) but in guard bees, which address the question of their role in the infection.

Thus, similar investigations of CBPV infection are now being conducted on other apiaries in an attempt to draw out the risk factors associated with CBPV expression.

Evolution of virus distribution in the colony and determination of CBPV genomic load level in correlation with CBPV expression are discussed

DEVELOPMENT OF A TAQMAN REAL-TIME TWO-STEP RT-PCR ASSAY FOR QUANTIFICATION OF CHRONIC BEE PARALYSIS VIRUS (CBPV) GENOME

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A two step real-time RT-PCR assay, based on TaqMan technology using a fluorescent probe (FAM-TAMRA) was developed to quantify chronic bee paralysis virus (CBPV) genome in bee samples. According to an absolute standard curve obtained with a plasmid containing a partial sequence of CBPV, this assay provided linear detection over a 7-log range (R^2>0.99) with a sensitivity of 100 copies, confirmed by reliable
inter-assay and intra-assay reproducibility. Standardisation including crushing, RNA purification and cDNAs synthesis was also validated.

In order to evaluate the CBPV TaqMan methodology, the CBPV genomic load was quantified in bee samples coming from an experimental infection by contact. Up to 1.9 x 10^{10} CBPV copies were revealed in a segment of insect body (head, thorax and abdomen) while lower CBPV genomic load was observed in dissected organs as mandibular and hypopharyngeal glands, brain and alimentary canal (up to 7.2 x 10^6 CBPV copies).

These preliminary results validate this method for chronic bee paralysis virus quantification.

THE CHRONIC BEE PARALYSIS VIRUS: A VIRUS LIKE NO OTHER; GENOMIC AND PHYLOGENETIC COMPARISON WITH OTHER BEE VIRUSES

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Most viruses of the honey bee are classified as “picorna-like virus”, in the Cripavirus genus (Dicistroviridae family) or in the Flavivirus genus. Chronic bee paralysis virus (CBPV) is a single-stranded RNA virus as “picorna-like viruses” are. However, CBPV has a multipartite RNA genome with two main RNA and differs especially from these viruses at genomic and phylogenetic levels. About 81% of the genomic sequence of the two main RNA have already been determined by different techniques such as cDNA library and 5’RACE. Sequencing is complexified by the apparent blockage of the 3’ end of RNA as some other RNA virus genomes are (Nodavirus, Tobamovirus or Bromovirus). Our first phylogenetic analysis, carried on nucleic sequence of the putative RNA dependant RNA polymerase, showed no significant similarity between CBPV and other honey bee viruses. However, few similarities between CBPV and viruses belonging to the Nodaviridae taxon (insect and fish viruses) have been shown.

The complete characterisation of the CBPV genus by sequencing and phylogenetic analysis is in progress.

TRANSMISSION ROUTES OF DEFORMED WING VIRUS

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Deformed wing virus (DWV) is a plus-stranded RNA-virus belonging to the floating genus Iflaviridae (insect and fish viruses) and could also be demonstrated in bumble bees recently. In most cases, DWV-infected bees do not exhibit overt signs of disease. In colonies infested by the ectoparasitic mite Varroa destructor DWV-infections can be associated with typical clinical symptoms like crippled wings, shortened and bloated abdomen, and discoloration. The recent publication of the genomic sequence of DWV was followed by the development of several RT-PCR protocols for the highly
sensitive and specific detection of this virus. Subsequently, numerous studies on the incidence and prevalence of DWV in honeybees and honeybee colonies were published. Still, little is known so far about the molecular pathogenesis of DWV. We investigated the first step in the pathogenic process, the transmission of the virus. We found evidence for vertical transmission of DWV through drones, horizontal virus transmission to larvae through feeding, and vectorial transmission to larvae and adult bees with Varroa destructor acting as possible virus vector. Interestingly, the occurrence of crippled bees correlated with the virus’ ability to replicate in mites. This phenomenon needs further investigation.

PREVALENCE OF SIX HONEYBEE VIRUSES IN BEEHIVES COLLECTED AT DIFFERENT AUSTRIAN LOCATIONS DURING DIFFERENT SEASONS, AND CORRELATION WITH NON-VIRAL DISEASES

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During May, July and September 2005 and in winter 2005/2006 honeybee samples were received from 23 Austrian apiaries. The samples were tested for Nosema apis and Malphighamoeba mellificae by light microscopy using half of the abdomens of 20 bees. The other parts of the bees were analyzed for the presence of sacbrood virus (SBV), chronic bee paralysis virus (CBPV), black queen cell virus (BQCV), deformed wing virus (DWV), acute bee paralysis virus (ABPV), and Kashmir bee virus (KBV) employing RT-PCR protocols published by BERÉNYI et al. (2006).

Malphighamoeba mellificae was detected only once (2%) in a sample taken during summer (July).

Nosema apis was found in 68% of the samples submitted during May, in 46% of the July samples, in 33% of the September samples and in 38% of the winter specimens.

Results for the presence of viral RNA are available until now for samples taken in May, July and September:

KBV as well as CBPV were never detected, while RNA of all other honeybee viruses investigated were identified in varying percentages:

ABPV: 2% (May), 57% (July) and 52% (September).
SBV: 44% (May), 72% (July) and 50% (September).
BQCV: 67% (May), 46% (July) and 33% (September).
DWV: 25% (May), 11% (July) and 24% (September).

THE FIRST RESULTS OF DIAGNOSTICS OF VIRAL DISEASES OF BEES IN SLOVAKIA

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Deformed wing virus (DWV) is the most commonly observed and best known honey bee virus in Europe. This virus was initially isolated from adult honeybees (Apis mellifera) from Japan. DWV is 30 nm isometric particles containing a single-stranded positive RNA, which is assigned to the genus Iflavirus. In recent study using molecular
techniques, was DWV detected in all life stages (eggs, larvae, pupae, adults) of honey bees and frequency of DWV-infected colonies was increased from spring to autumn. DWV can cause morphological deformities, as are: wing deformity, malformed appendages, shortened abdomens and miscolouring.

DWV generally persists as a latent infection with no apparent symptoms. In adult bees, during the year DWV was found at least once in 97% of the apiaries and for pupae it was 94% of the apiaries. The question is, what is reason of change from a latent infection to productive infection, which results in collaps of all colonies.

The disease and mortality caused by DWV have been associated with severe infestation of the ectoparasitic mite Varroa destructor. This mite is the most commonly observed parasit of bees in the world. Generally, two hypotheses are cited that could explain the deleterious effects of varoa parasitism on honey bee colony decline. First, the mite can act as a vector and can directly inject virus particle into the insect hemolymph. Second, the mite can also trigger virus replication by a simple mechanical effect, cuticle piercing, or by injection of external proteins into the insect hemolymph. The latter hypothesis is supported by several studies that demonstrated that there was reactivation of viruses already present in the insect following experimental inoculation.

Several techniques have been employed for detecting bee viruses, including indirect fluorescent-antibody analysis (IFAT), agarose gel immunodiffusion, an enzyme-linked immunosorbent assay (ELISA), Southern hybridization, reverse transcription-PCR (RT-PCR) and real-time quantitative RT-PCR.

RECIPROCAL SEQUENCE EXCHANGE BETWEEN NON-RETRO VIRUSES AND HOSTS LEADING TO THE APPEARANCE OF NEW HOST PHENOTYPES

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Divergence among individuals of the same species may be linked to positional retrotransposition into different loci in different individuals. Here we add to recent reports indicating that individual variance occurs due to the integration of non-retroviral (potyviral) RNAs into the host genome via RNA recombination followed by retrotransposition. We report that in bees (Apis mellifera), approximately 30% of all tested populations carry a segment of a dicistrovirus in their genome and have thus become virus-resistant. Reciprocally, segments of host sequences have been found within defective-interfering-like sequences of a dicistrovirus. Similarly, host sequences were found fused to potyviral sequences, previously described integrated into their host genome. A potential, continuous RNA exchange leading to divergence is discussed.
Demonstration of Temperate Bacteriophage as a Lytic Agent of Paenibacillus larvae subsp. larvae Culture in MYPGP Medium

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The cause of lysis of Paenibacillus larvae subsp. larvae in/on MYPGP broth/agar without sporulation was not understood. This property is important to design an appropriate sporulation medium and to study of secreted products of this bacterium. We tested lytical activity of secreted molecules of P. larvae subsp. larvae on other bacteria (Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Enterobacter cloacae and Escherichia coli) and on the same strain of Paenibacillus larvae subsp. larvae. Simultaneously, the presence of bacteriophage in lysed culture was investigated by using direct electron microscopic methods and by isolation of phage DNA. The lytical activity of cell-free lysate on other bacteria and on fresh culture of P. larvae subsp. larvae was not found, but the bacteriophage particles were detected in high concentration in lysed culture. The results of isolation of phage DNA was not significant, because the phage ghosts in the lysates predominated. The induction of temperate bacteriophage was started by the decrease of pH below 6.4 and by the accumulation of metabolites in the culture. The bacteriophage was identified as BLA or PBL1 according to morphological properties, because its DNA sequence is not known.

Nosema Disease in European Bees

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Currently, three species of microsporidia from the genus Nosema have been identified in European bees, namely Nosema bombi from bumble bees (Bombus spp.) and Nosema apis and Nosema ceranae from the honey bee Apis mellifera. Using molecular genetic approaches (DNA sequences of the rRNA gene), N. bombi has been positively identified from a wide range of bumble bee species (Tay et al. 2005). It was formerly considered that N. apis was host-specific to A. mellifera and that N. ceranae was a parasite of the Asiatic honey bee Apis cerana. However, we and a number of other
labs have recently confirmed the presence of N. ceranae in European honey bees. We have developed molecular genetic markers to allow rapid and sensitive discrimination among these three Nosema species and have used them to document the presence of Nosema among European bees. We thereby aim to shed light on the origins and epidemiology of Nosema among European bees.


**DISEASE REMOVAL BY ALTERED FLIGHT BEHAVIOR OF FORAGER HONEY BEES (APIS MELLIFERA) INFESTED WITH NOSEMA APIS**

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Varroa destructor, a major pest of honey bees, alters flight behavior of foragers to a degree that may prevent their successful return to the colony. Infested workers have impaired orientation and need more time to return to the colony. As V. destructor is a relatively recent pest of the western honey bee Apis mellifera and thus specific adaptations cannot be expected, it would be possible that the behavioral response is more general in nature. Here we tested the flight behavior of foragers infested by Nosema apis. Sampling departing and returning bees at the entrance showed lower infestation of returning workers compared to departing workers indicating higher loss of infested bees. Infested workers took 1.7 times longer to return to the colony than uninfested workers when released from the same location. Prolonged flights were also confirmed by labeling workers with radio frequency identification tags (RFID) to register their departure and return at the colony entrance. This data also showed that infested workers had shorter life spans and vanished from the colony earlier. The study shows a similar effect of parasitism on flight behavior of foragers infested by N. apis to that shown for V. destructor suggesting that the altered flight behavior of foragers is a general response by diseased bees and it is not limited to infestation by V. destructor. This behavior can be interpreted as suicidal pathogen removal, serving as a disease defense mechanism which reduces the colony’s load of parasites or pathogens.

**CROSS INFECTIVITY AND IMPACT OF NOSEMA BOMBI ON COLONY DEVELOPMENT OF BOMBUS TERRESTRIS**

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A Nosema bombi infection can be induced via administration of N. bombi spores to host brood. The open larval stage is the brood stage in which a specific amount of spores can be administered artificially. Cross infection studies were conducted by individual administration of 5 µl of a N. bombi spore suspension in sucrose-solution
12.5% (w/v) to larvae in open cells. The adults that emerged from these cells were diagnosed within 24 hours after emergence. Administration of N. bombi spores from both B. pascuorum (250 000 spores per larva) and B. hypnorum (500 000) did not result in a microscopically detectable infection whereas comparable numbers of N. bombi spores for B. terrestris did result in an infection. The impact of N. bombi on colony founding and transmission was studied by inducing infection with N. bombi spores from B. terrestris adults to individual worker and queen larvae in open cells of B. terrestris colonies. Administration of 312 500 spores to individual queen larvae resulted in a N. bombi infection in part (30%) of the queens. Mating and hibernation were not affected but colony founding was impeded. Molecular diagnosis (ITS-f2/r2) of N. bombi in queen fed with spores showed N. bombi in part of the queens, in both the intestines and ovaries or only in the ovaries. Transmission in a colony was studies by infecting worker larvae. During colony development age cohorts were marked: adults that emerged before spores administration, adults that were supplied with N. bombi spores in the open larval stage, adults that were in the egg stage when the spores were supplied and adults that were ovipositioned 11 days after spores administration. Infection developed in the workers treated in the larval stage and was transmitted to the future age cohorts in the colony and to the adults that were in the colony before the introduction of the N. bombi infection. The brood nests of the Nosema-infected colonies were smaller than of the non-infected colonies.

**SPREADING OF THE CAUSATIVE AGENT OF EUROPEAN FOULBROOD IN BEE COLONIES BEFORE AND AFTER THE SANITATION OF AN INFECTED APIARY**

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European foulbrood (EFB) newly infested apiaries have strongly increased during these last years in Switzerland. Therefore it is questionable if the control measures implicating the destruction of colonies with clinical symptoms, the burning of the brood material and the disinfection of beehives are sufficiently effective to maintain the bacterium Melissococcus plutonius, the causative agent of EFB, under the threshold damage.

In order to quantify the amount of M. plutonius in bee samples, a novel real-time PCR system has been established. Testing this system with samples analysed previously with the hemi-nested PCR system developed by Djordjevic and co-workers (1998) gave results totally in accordance with the hemi-nested system, thus confirming the specificity of our system towards M. plutonius.

For this study, 14 infected apiaries have been investigated. Samples were taken before the sanitation and later, in autumn before the wintering period and the following spring. Bee samples have been collected in brood nest and hive entrance from up to 8 colonies per apiary. Following DNA extraction, the samples were subjected to real-time PCR analysis.

Results show that the amount of M. plutonius has markedly decreased after the sanitation of infected apiaries but not all colonies could be brought to the wintering period free (i.e detectable amounts) of EFB. It is also noticeable that hive entrance bees were less infected than brood nest bees.
**Changes in the Bee Pathology Chapter of the New Edition of the OIE Manual**

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Due to transport of bees and bee products across borders and continents, an increasing spread of pathogenic agents was observed. Therefore, diagnostic laboratories have to update their diagnostic techniques on a regular basis. The International Animal Pest Code of the OIE denoting the status of individual countries or regions concerning the existence of bee diseases as well as the transport of bee colonies within the respective regions requires a harmonisation and standardisation of diagnostic methods. Therefore the chapters on bee diseases of the “OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals” have to be updated from time to time.

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**Nosema Ceranae and Nosema Apis in France: Co-infections in Honey Bee Colonies**

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Nosemosis is a severe pathology caused by the microporidian Nosema spp. So far only two microsporidian parasites have been described in honey bees: Nosema apis and Nosema ceranae. N. apis has been detected in European honey bees (Apis mellifera) and was one of the earliest described microsporidians (Zander, 1909). N. ceranae has been initially described in Asian bee colonies (Apis cerana) and was recently found in A. mellifera colonies in Taiwan and Europe. Very few data on this parasite are available, especially on its distribution, while Nosema apis is known to be worldwide distributed. Information on symptoms resulting from N. ceranae infections in A. cerana or A. mellifera colonies are limited. On the opposite pathology symptoms due to N. apis are well documented.

Cross infections between the two host species have demonstrated that N. apis is infective in A. cerana, although its development is less effective in the Asian host compared to the European one. Spores produced by the two Nosema species are quite similar and can hardly be distinguished by traditional light microscopy analysis. In this work PCR techniques were used in order to characterise infections or co-infections by these two pathogens in French honey bee colonies. Samples were collected in France, PCR analysis were run in Spain. A total of 46 honey bee samples were analysed. Prior PCR analysis, spores were visually detected in samples. Samples have been collected during the years 2003, 2004 and 2005. The first results have shown that N. ceranae was well distributed in France: sequences of N. ceranae were found in different places distant from hundred kilometres. Coinfection with N. apis and N. ceranae were demonstrated in 3 samples. All these results show that N. ceranae has been present in France for several years.
A NEW APPROACH FOR AMERICAN FOULBROOD PREVENTION

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American Foulbrood (AFB) is the most widespread and destructive brood diseases caused by the spore forming, aerobic bacteria Paenibacillus larvae subsp. larvae. The occurrence of this disease has considerable economic impact on the bee industry, as well as valuable in terms of pollination and production of honey and beeswax. The best treatment for this disease is the destruction of hives by burning, but some Countries authorised the use of antibiotics, like oxytetracycline and tylosine, to treat infected hives. Unfortunately, sometimes these treatments were incorrectly applied to the treatment of AFB development causing spreading of antibiotic resistance and pollution of honeybees products. Lactic Acid Bacteria (LAB) represent a new valuable tool for the prevention of AFB disease. They are normal inhabitants of human and animal gut and are also been found in adult and larvae of honeybees, with particular reference to lactobacilli. Some studies demonstrated that some microbes, belonging to the genus Lactobacillus, can develop beneficial actions on gastro-intestinal diseases prevention and can contribute to maintain host’s health. We previously found that some LAB, normal inhabitants of honeybees gut, may inhibit in vitro the growth of AFB- and EFB-causing bacteria. The aim of the present study, was the investigation of the possibility to in vivo prevent AFB development in honeybees by the use of selected probiotic bacteria. We selected healthy colonies to infect artificially and made three group:

A – control (non treated);
B – treated group with probiotic administration before the infection;
C – treated group with probiotic administration immediately after the infection.

We observed the presence on combs of diseases symptoms as concave and dark capping, dead larvae or scales and monitored the presence of Paenibacillus larvae and our probiotic on larval gut by mean of microbiological counts. We observed a decrease of disease symptoms on treated hives.

THE INCIDENCE OF HONEYBEE PARASITES AND DISEASES IN TURKEY

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Parasitic mite, Varroa destructor is well known parasite of honeybee and was detected in almost all apiaries that were sampled in Turkey. However, varroa mites were not detected in some colonies. Therefore, some levels of resistance to varroa might be developed in the endemic populations over time. On the other hand, there are open debates about the existence of tracheal mites, Acarapis woodi in Turkey. Interestingly, A.woodi was not found from large no. of workers sampled in most regions of Turkey even though it was reported in most of the neighbouring countries (Ellis and Munn 2005).

Nosema (Nosema apis) and Amoeba (Malpighamoeba mellificae) disease level is usually higher in Blacksea and Marmara region more than other regions. Precipitation

31
but not the temperature was a significant factor for predicting nosema in wet regions. No data has been reported about Nosema cerana cases in Turkey yet. Brood diseases, AFB (Paenibacillus larvae larvae) and EFB (Melissococcus plutonius) are not widespread and only very few cases were reported (Simsek & Ozcan 2001; Ozkirim & Keskin 2002). Chalkbrood (Ascopaera apis) was transmitted to Turkey by imported wax in 1986 and did spread all over Turkey.

Wax moth (Galleria mellonella) is common and becomes a major problem in some years particularly in Egean and Mediterranean regions. Braula is seldom seen and small hive beetle (Aethina tumida) has not been detected yet. There is not much known about honeybee viruses in Turkey. Bee paralysis or “hairless black syndrome” is the most common case of virus disease in Anatolian bees reported by Ruttner (1988).

FIRST STEPS TOWARDS THE IN VITRO CULTIVATION OF NOSEMA CERANAE

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Microsporidia are known to infect nearly all animal phyla, but insect microsporidia are the commonest. Although the transmission of insect microsporidia to mammals is improbable, it has recently been considered possible. Nosema ceranae has recently been recognized as a microsporidian pathogen of the honey bee Apis mellifera. Establishment of an in vitro propagation system would enable pathobiological studies of this parasite. The production of large numbers of spores would allow the development of different types of in vitro and in vivo studies, including improvement of diagnostic methods by production of monoclonal and polyclonal antibodies.

For this purpose, we have infected Vero-E6 cell with N. ceranae spores obtained from an autochthonous natural infection. Infected cells were cultured under different conditions. Most developmental as well as spore-forming stages were observed around the nucleus. Differences in growth rate were observed related to growth temperature. Results will be discussed.

Future experiments with different cell lines and culture media are needed to improve the harvest rate of spores. However, these preliminary experiments show the capability of N. ceranae to develop in in vitro culture. Furthermore, as mammal cells were infected at 37°C, this opens up the possibility of them being a source of human microsporidiosis.

HONEYBEE DISEASE CONTROL IN THE EU: THE COMMISSION’S DECISION FOR GRANTING EXEMPTIONS TO THE PRESCRIPTION-ONLY PRINCIPLE

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Under the new EU veterinary pharmaceutical legislation, all veterinary medicinal products used in food-producing animals will require a medical prescription from 1 January 2007. According to the commission’s draft decision exemptions could be
AN APPROACH TO NOSEMA CERANAE CONTROL WITH FUMAGILLIN IN FIELD CONDITIONS

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The honeybee nosemosis incidence is increasing significantly in Spain for the last years and the recent detection of Nosema ceranae could be related with this fact. Fumagillin has been shown effective in Nosema apis treatment (Katznelson and Jamieson, 1952). However, no data are available about the N.ceranae control with this antibiotic or its capacity to inhibit parasite intracellular development.

A total of 20 hives randomly collected were analyzed to detect pathogens presence. Bee samples came from 14 apiaries belonging to 3 professional beekeepers (with around 2,000 hives distributed in east and central Spain), who had observed a clear signs of population depletion and mortality of colonies, and a decrease on honey production like the only symptoms. A high Nosema spp spores level were observed in all the samples and the specie was confirmed as Nosema ceranae by molecular diagnosis (Higes et al., 2006).

A total of 120 mg of fumagillin (CEVA) per hive was administered, dividing the total dose in four treatments once a week. The antibiotic was dissolved in 500 ml of water/sugar solution at equal quantities (w/v). Bee samples were taken one week after the third and fourth treatment, from the same colonies and again analyzed to detect Nosema spores. Three samples were on positives after the third one (light microscope) and no one after the fourth treatment (confirmed afterwards by PCR method).

Any adverse effects were not observed after the antibiotic administration and the beekeeper notify a satisfactory colony evolution.
EFFECTS OF NATURAL COMPOUNDS ON NOSEMA DISEASED HONEYBEES IN LABORATORY CONDITIONS

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Nosemosis is one of the most widespread honeybee diseases, caused by the microsporidian Nosema apis. The only effective substance against this parasite, fumagillin, is no longer available for use in most European countries. For this reason, the potential of some natural compounds for the control of Nosema infection in honeybees was investigated.

Newly emerged adult bees were artificially inoculated with a known amount of N. apis spores by force-feeding the bees with a sucrose suspension. Groups of 30 bees were kept in small cages for 25 days and were fed with candy treated with thymol, resveratrol, vetiver oil, lysozyme or with untreated candy. Two randomly chosen individuals were collected from each cage at different times and examined to measure the progress of the infection; the number of dead and living bees was recorded every 3 days. From the results it emerged that thymol and resveratrol were the most promising in effectively reducing Nosema infection.

RELIABILITY OF DIAGNOSTIC METHODS TO DETECT NOSEMA SPP. SPORES IN HONEY BEES: MOLECULAR IDENTIFICATION VERSUS VISUAL OBSERVATION

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Two direct methods to detect Nosema ceranae in Apis mellifera were tested to establish the reliability of diagnosis. A total of 87 bee samples were analyzed by visual observation of Nosema spores by way of the OIE recommendations as well as by PCR and posterior sequenciation. To visualize the spores, the abdomens of ten old honey bees from each sample were macerated in 5 ml of distilled water. The suspension was filtered and a second 5 ml of water used to rinse. The suspension was centrifuged for 6 min at 800 g. The pellets were resuspended again in 1 ml. Spores were identified under phase contrast microscope (x400) in 0,1 ml of the sediment.

Molecular diagnose required previous spore germination that was induced in all the samples with the following profile: 0,5 ml of the resuspended pellet of each sample was mixed with 200 µl of freshly prepared germination buffer (0.5M sodium chloride, 0.5M sodium hydrogen carbonate, pH to 6.0 with orthophosphoric acid; De Graaf et al., 1993) and incubates at 37º C for 15 minutes allowing spores germination (Rice, 2001). DNA extraction, PCR amplification and sequencing of the 16S rRNA sequence were done as
described previously (Higes et al., 2006) in all the samples. PCR positive samples were 81.6% while only in 53.3% samples spores were MO observed (sensitivity 69%; specificity 100%). Only 5 samples were identified as Nosema apis and 82 were Nosema ceranae.

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Influence of sampling in the detection of Nosema ceranae spores

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An infected hive parasitized with Nosema ceranae (molecular and morphologically characterized) was analyzed by visual observation of Nosema spores by way of the OIE recommendations during spring and summer months. Bee samples were taken in the morning at 8,30 h and 12,30 h. Hive entrance was sealed for half an hour; bees arriving were collected and sample marked as exterior bees. Lately another group of bees were brushing from combs close to breeding nest, and sample marked as internal bees.

The abdomens of ten honey bees from each sample were macerated in 5 ml of distilled water. The suspension was filtered and a second 5 ml of water used to rinse. The suspension was centrifuged for 6 min at 800 g. The pellets were resuspended again in 10 ml. Spores were counted under the cover-slip of a haemocytometer. In a second trial, 30 interior and 30 exterior bees collected the same day at 12,30 h were individually analyzed using the same method.

Parasite burden measured by the number of spores per bee are clearly influence either by the type of bee as well as the time of sampling. Exterior bees presented a much higher level of infection (10 fold) than the interior bees, usually collected to diagnose nosemosis. Both interior and exterior bees had almost double quantity of spores when collected at 12,30h than at 8,30 h.

In the second trial, only 15% of interior bees were positives while 50% of the exterior ones.

It is concluded that exterior bees collected at noon are a more reliable sample in spring and summer.

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EXPERIMENTAL INFECTION OF APIS MELLIFERA HONEYBEES WITH NOSEMA CERANAE SPORES

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In this report, we describe an experimental infection of Apis mellifera by Nosema ceranae, a newly reported microsporidium.

Newly emerged Nosema free honeybees were inoculated with 125,000 Nosema ceranae spores per bee, collected from live heavily infected bees.

Three replicates cages of 20 honeybees each were done and one control cage (n=20) was done additionally which received 10µl of plain sucrose solution.

Each cage was checked daily and any dead bees counted and removed. One bee of every group was collected 3h, 3, 6 and 7 days p.i. and ventriculus prepared for MO and ME study.

Control bees were negative all through the study. Epithelial morphology showed no alterations due to methodology. Only one bee died on day 7 p.i.

In the infected groups, mortality was not observed until day 6 p.i. (33,3% ± 5%). In the three infected replicates, 94,1% bees died on day 7 p.i.

At 3 hours p.i., ventriculi looks healthy and only mature spores were observed in the intestinal lumen. Although some spores appeared empty, infected cells were not observed at that moment.

On day 3 p.i., ventriculi appeared whitish and shrunken; epithelial cells of samples from the three replicates were infected. Only the superficial epithelial layer presented all the intracellular parasite stages of the endogenous life cycle. Infected cells filled with different parasitic stages were scarce and surrounded by healthy uninfected ones. Emptied spores were observed inside infected cells.

On day 6 p.i., all the epithelial layers were affected in three replicates. Superficial layers were loaded with more mature stage.

EFFECT OF SANITIZING AND CURATIVE TREATMENTS ON THE INFESTATION OF BEE Colonies BY THE SPORES OF PAENIBACILLUS LARVAE SUBSP. LARVAE

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The objective of the study was to assess the health status of all bee colonies in an apiary in which clinical symptoms of American foul brood were found and to examine the impact of different sanitizing and curative treatments on the infestation level by Paenibacillus larvae larvae of bee colonies in the subsequent beekeeping season. The study was run in the years 2004-2005 in a 26-colony strong apiary. In 2006 upon inspection 3 colonies tested positive for the American foul brood. From the diseased colonies comb sections with lesioned brood were taken for diagnostic tests and from all remaining colonies both honey samples and bee samples were taken in order to test them for the spread of the disease. As a result of microbiological tests P. l. larvae was isolated.
and identified in 29 bee colonies. Depending on infestation level by the spores of P.l. larvae (number of bacterial colonies isolated on growth media). The colonies were divided into groups and subjected to different disease control treatments.

   Group 1 – colonies with clinical symptoms of American foul brood and colonies highly infested with P.l. larvae transferred to clean hives with foundation comb and fed an antibacterial formula administered in sugar syrup.
   Group B – colonies medium-highly infested not transferred but given syrup with an antibacterial formula
   Group C – low infested colonies subjected to no sanitizing or curative treatments
   Group D – colonies not found positive for P.l. larvae – not subjected to any sanitizing or curative treatments

In the beekeeping season of 2005 colonies were resampled for honey and bees to be tested for the presence of P.l. larvae.

From among 10 bee colonies of group A, resettling and treatment with curative formula notwithstanding, 5 colonies died by May of 2005. Of the surviving colonies, three still tested positive for P.l. larvae but the infestation level substantially declined. Only in 2 colonies of that group no P.l. larvae was found. In the group which was subjected to curative treatment only (B) two colonies died, their infestation level rising to high. Likewise, in three out of the remaining 8 colonies of that group an infestation level by P.l. larvae higher than that in 2004 was found whereas in 5 colonies infestation stayed at the same level. In the colonies of Group C in which the infestation level was low and hence they were subjected to no treatments there were also losses amounting to 3 colonies. The laboratory tests confirmed infection by P.l. larvae in four colonies that stayed unchanged from 2004, in one of them clinical symptoms having occurred and two colonies testing negative for the presence of P.l. larvae.

In group D, free of infestation in 2004, the following season the presence of P.l. larvae was found in 2 colonies.

Based on the tests performed it can be stated that in colonies in which clinical symptoms of American foul brood occur or in which the infestation level by P.l. larvae is high not even resettlement or treatment with antibacterial drugs can yield the results expected. The presence of colonies sub-clinically infested with American foul brood enhances infestation level of the colonies as well as promotes further spread of the disease.

**IDENTIFICATION OF VIRULENCE FACTORS OF P. LARVAE**

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Paenibacillus larvae (P. larvae) is the causative agent of American foulbrood, a globally spread bacterial disease of honey bee brood. AFB is highly contagious and able to kill infected colonies. Despite its disastrous economic impact on apiculture little is known about the molecular pathogenesis of this disease. Here we report first attempts to identify virulence factors of P. larvae using comparative genomics. By serially passaging a virulent wild type strain of P. larvae on nutrient agar we obtained an attenuated laboratory strain of P. larvae. In exposure bioassays this attenuated strain revealed both,
a higher LC50 and a higher LT50 than the wild type strain. Hence, its virulence was reduced. When the genomes of both strains were compared by using the method of subtractive suppression hybridization (SSH) four candidate genes presumably involved in virulence determination were identified. Further studies will characterize these genes in detail and evaluate their role in the molecular pathogenesis of P. larvae.

SPREAD OF AMERICAN FOULBROOD THROUGH AETHINA TUMIDA?
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American foulbrood is a serious disease of honeybeebrood. The disease is caused by the spore-forming bacterium Paenibacillus larvae. Small hive beetles (Aethina tumida) and his larvae feed on honey, pollen and beebrood. As the beetle is a very active and persistent flyer, we examined if contamination of spores of Peanibacillus larvae through Aethina tumida is possible. First results will be presented.

QUANTITATIVE METHOD FOR THE DIAGNOSIS OF HONEY-BEE NOSEMOSIS
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The incidence of nosemosis remains at high level in Romania. Infections with Nosema apis (Zander) are acquired by the uptake of spores during feeding or grooming; also clinical signs and pathological modifications of the adult honey-bees are common with other diseases. There is presented the National Surveillance Program for Nosemosis at bee colonies.

There is established a standardized procedure in order to obtain the accuracy necessary for a diagnosis, according with the principles of the quality system. The suitable laboratory protocol assures the real and representative determination of the infectious level in bees, with Nosema spores.

The method principle is to determine the protozoan spores from bees triturate and to count them with a Bürker-Türk hemocytometer.

The paper presents the principal phases and the evaluation of the performer characteristics of the method: specificity, reproducibility, precision and incertitude of measure, compulsory parameters for to validate this procedure.

Finally, it has been established 3 principal infectious levels.
ACTIVATION OF NOSEMA APIS SPORES BY CARBON DIOXIDE

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Carbon dioxide (CO₂) can be used to disinfect honeycombs against the most harmful pest of the honeycombs, the greater wax moth (Galleria mellonella L.). However, it is not known, how this gas affects Nosema apis which are one of the most common parasites on the honeybee. The aim of this study was to examine if treatment of N. apis spores with CO₂ effects their ability to infect honeybee workers.

An inoculating solution was prepared and divided into 4 doses. Three doses were exposed to the effect of CO₂ (100% concentration), for 30, 35 and 40 hours. The fourth dose was used as a control, without exposure to CO₂. Each of the four doses was used to individually inoculate 120 workers from each group. The fifth group was formed of workers that were not inoculated with spores of N. apis. The cages with the bee workers were kept in an incubator, under 30°C. The course of invasion by parasites was tested every third day, by counting the number of N. apis spores in the digestive tracts of living workers.

The significant differences in the rate of infection were observed from the 9th day after inoculation. Between groups there were significant differences in the survival rates of workers. Treatment of the N. apis spores with CO₂ resulted in a faster proliferation of the parasite and higher mortality among the workers.

MOLECULAR SCREENING BY PCR OF HONEY BEE LARVAE FOR MELISOCOCCUS PLUTONIUS INFECTION

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European foulbrood is a disease of honey bee larvae caused by Melisococcus plutonius and secondary bacterial invaders. The diagnosis is usually performed through bacteria isolation which can be hampered by particularly stringent growth conditions requirements and competition with other microbes.

PCR assay can be an useful, high sensitive and specific method to assays the honeybee larvae. We analysed several honey bee larvae and few isolated bacterial strains, by an amplification of a DNA fragment of 16S ribosomal RNA gene. The original protocol of Govan et al. had been modified to obtain a higher sensitivity. All bacterial strains isolated by infected honey bee larvae resulted positive, while several other bacterial DNA resulted negative.
Purification and biochemical characterization of proteolytic enzyme of bacterium Paenibacillus larvae subsp. larvae

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Introduction: Bacterium Paenibacillus larvae subsp. larvae is the most dangerous pathogen of bees due to an extremely resistance of its spores. It secretes several proteolytic enzymes during its growth, but their role in pathological processes as well as their biochemical characterization are still unclear. The results of biochemical research of these enzymes might explain their role in the pathogenesis, but it is necessary to use a purified material.

Methods: For production of proteolytic enzymes of bacterium Paenibacillus larvae subsp. larvae, The MYPGP medium was used. The basic biochemical information about these secreted enzymes were obtained by zymography. The optimal incubation conditions and inhibition pattern were detected by the same method. Inhibitors of all classes of proteolytic enzymes were used (Pepstatin, Leupeptin, E64, Iodoacetamide, PMSF, EDTA, EGTA a 1,10-Phenantroline). Gel filtration (Sephadex G-200) and ion exchange chromatography (DEAE cellulose) were proposed to purification of these enzymes.

Results: In the cell free supernatant, the proteolytic enzymes with approximately molecular weight of 87, 74, 40 and 42 kDa were detected. All of these enzymes were inhibited by inhibitors of metaloproteases, but not by inhibitors of other classes. pH optimum appeared about pH 7.0 with some differences between 87/74 kDa and 40/42 kDa enzymes. Proteolytic enzymes may be optimally purified after 24 hrs of incubation at 4°C by gel filtration followed ion exchange chromatography. Detected enzymes probably have character of exoproteases according to substrate specificity analyses.

Conclusion: We have found that the bacterium Paenibacillus larvae subsp. larvae secrets two different classes of proteolytic enzymes which probably are metalloproteases. Their exopeptidase character indicates their role in nutrition of this bacterium. Suggested purification procedure may be used to obtain of purified enzymes.

Survival of American foulbrood pathogen in hot beeswax

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The most significant problems at the American foulbrood (AFB) control are due to the resistance of the AFB pathogen, the sporulating microbe Paenibacillus larvae. We studied the possibility of the beeswax disinfection. The experiments were based on the beeswax analysed as P.l. free. Into this beeswax there were inserted quantitatively P.l. spores (stem 643/04) obtained from the scales of the AFB combs in the quantity 9.10⁷ CFU/g beeswax.

For the thermal exposition oil bath was assembled with submergible heater and oil air mixer. The bath temperation was controlled by calibrated thermocouple and the achieved temperature oscillation in the whole bath volume was less than 0,05 °C Elected temperatures: 90-108-121-134 °C, applied time expositions: 0,3-1-2-4-6-12-24 hours
After the thermal expositions the wax was taken into warm physiological solution and after thorough agitation and cooling the agar plates were inoculated and subsequently cultivated. The growing colonies of *P. l.* were re-counted per 1 g wax after five days.

The spores of AFB pathogen are very resistant to temperatures in the standard processing of beeswax. As to achieve safe reduction of the viable spores of *P. l.* more hours heat of beeswax at the temperature more than 130 °C is necessary. But the long time heat of wax changes also its chemical characteristics.

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**THE USE OF COMMERCIAL NUTRIENT AGAR TRIOS FOR DIAGNOSIS OF AMERICAN FOULBROOD IN HONEYBEES**

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In the programs of the American foulbrood control there is very important in time exact diagnosis of the incidence of sporulating microorganism Paenibacillus larvae. Samples for the diagnostics are taken from various material (brood, combs, debris, honey). After the sample preparation they are cultivated on agar plates. In the accredited laboratories in the Czech Republic the cultivation is carried out on MYPGP agar prepared by laboratories by themselves. Ring test has shown that one of the variability sources is the nutrient agar prepared in small charges in individual laboratories.

On the basis of cultivating medium developed in the Bee Research Institute Dol the commercially prepared medium MKM™ MYPPN was introduced into serial production.

Validation trials have shown that the medium is suitable for routine laboratory diagnostics.

The use of the commercial medium MKM™ MYPPN is for small laboratories more cost effective than laboratory preparation of this nutrient medium.

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Macroparasites

Symposium organized by Peter Neumann

PROXIMATE MECHANISMS OF SOCIAL PARASITISM BY HONEYBEE WORKERS, *APIS MELLIFERA CAPENSIS* ESCH.

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Cape honeybee workers, *A. m. capensis*, are facultative social parasites and can have a dramatic impact on queenright colonies of other subspecies such as the neighbouring one, *A. m. scutellata*. In South Africa, this “dwindling colony syndrome” is caused by a single clonal lineage, which we here use to study the underlying proximate mechanisms of social parasitism by workers. Initially infesting workers can easily overcome the inhibitory pheromone secretion of the host queen and brood, because 94% of them develop into pseudoqueens. However, the reproductive development of their own offspring is inhibited (3.1%), suggesting that the parasitic pseudoqueens together are more efficient than the host queen at regulating reproduction. Indeed, in paired arena tests, single parasitic workers were pheromonally dominant over all other worker groups and even over *A. m. scutellata* queens. This indicates an outstanding ability for pheromonal competition, because the reproductive division of labour in this highly eusocial species can be challenged. Thus, it appears as if the ability to both overcome and produce inhibitory pheromones is crucial for social parasitism by honeybee workers.
FORENSICS OF ABANDONED HONEYBEE NESTS: REPRODUCTION OF SMALL HIVE BEETLES (AETHINA TUMIDA) AND GREATER WAX MOTHS (GALLERIA MELLONNELLA)

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Abandoned honeybee nests can serve as a nutrition source and breeding substrate and habitat for a variety of arthropods as small hive beetles (=SHB), Aethina tumida, and greater wax moths (=GWM), Galleria mellonella. Differences in preparation for absconding between European and African honeybee subspecies may influence the reproductive success of these pests. Absconding African colonies (N=14) left significantly less brood and stores behind than European ones (N=19; p<0.05), while SHB reproduction was significantly reduced by the preparation efficacy for protein sources of the respective honey bee subspecies (pollen: rₛ=0.9, t=4.3, p=0.008; brood: rₛ=0.9, t=3.7, p=0.014), this was not the case for GWM reproduction, which occurred even on empty old brood combs. SHB reproduced significantly less often in abandoned African nests (0%) than in European ones (63%; p=0.001), while there were no significant differences in the frequency of reproduction of the moths (27% and 63%; p>0.05). Both pests tended to negatively influence the reproductive success of each other. There was also a negative influence of ants on SHB reproduction. In contrast to moth larvae, SHB larvae did not completely destroy the combs. Consequently, moths could still reproduce in nests after SHB mass reproduction, but not the other way around. We conclude that the preparation efficacy for absconding has a major impact on SHB reproduction and may contribute to both the invasion success of SHB in its new ranges.

BUMBLEBEES AND STINGLESS BEES AS ALTERNATIVE HOSTS OF SMALL HIVE BEETLES


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When parasites of social insect bees become invasive species, they may cause severe damage to non-sympatric hosts. The small hive beetle (SHB), Aethina tumida, is a parasite and scavenger of honeybee, Apis mellifera, colonies native to Africa and has a higher impact in populations of European-derived hosts in North America and Australia. Frequent collapses of European-derived colonies probably result from quantitative differences in a range of behaviours between African and European honeybee subspecies. Thus, the higher susceptibility of European-derived honeybees, being itself an invasive species in North America and Australia, seems to facilitate the spread of SHB, another invasive species. Bumblebees and stingless bees naturally occur in the new SHB ranges and share important features with honeybees, suggesting that a host switch...
from honeybees may occur. Experiments were conducted using colonies of Bombus impatiens and Trigona carbonaria. The data show that both bumblebee and stingless bee colonies are attractive to free-flying SHB, indicating that they can actually serve as alternative hosts. However, behavioural resistance mechanisms were found in both B. impatiens and T. carbonaria. This suggests that general behavioural defence mechanisms against nest intruders also seem to provide protection against invasive species. Nevertheless, our results indicate a potential for an invasion meltdown in social bees. The actual impact of this on native biodiversity will depend on the infestation levels of bumblebee and stingless bee colonies by SHB in the field. It will also depend on the impact of SHB on feral honeybees in Australia and America.

**BEEKEEPERS' OBSERVATIONS ON THE SMALL HIVE BEETLE (AETHINA TUMIDA) AND OTHER PESTS IN BEE COLONIES IN WEST AND EAST AFRICA**

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Since the recent introduction of the African small hive beetle *Aethina tumida* (Coleoptera, Nitiludae) into other parts of the world it has become relevant to gather more information about hive beetles and other pests in subsaharan Africa where they are indigenous. Until now most records are from South Africa, from beekeepers and bee researchers, but there are some reports from other areas as well.

The opinion exists that the small hive beetle is less noxious in their area of origin than in most countries where they have been introduced recently. But there are beekeepers in Africa who consider the pest as a serious threat to colony development and honey quality. In West Africa the small hive beetle seems to be a bigger problem than in East Africa or the Horn of Africa. Ecological conditions such as climate and soil humidity, but also the occurrence of predators and parasites may differ regionally.

The word hive in the name hive beetle may suggest that the beetles occur mainly in man-made or man-provided housing for honeybee colonies, but the word hive is also used for feral colonies of honeybees hosting them. Besides there are also stingless bee species, for example *Dactylurina staudingeri* (Meliponini) which are hosts to the small hive beetle.

**MALE FITNESS IN RELATION TO COLONY DEVELOPMENT AND VARROOSIS INFECTION**

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Numerous investigations confirm strong effects of Varroa destructor infestations on colony development and the viability (flying ability, life expectancy, number of spermatozoa etc.) of *Apis mellifera* drones. For a better understanding of natural selection processes and with regard to the selection of Varroa tolerant bee stock we want to estimate the influence of Varroa infestation on the male mating success of bee colonies.
In 2005, we used the island mating station of Norderney to compare the colony development and mating success of 26 widely untreated sister colonies under isolated conditions. The strength of colonies, the extend of drone brood, the number of adult drones, the Varroa and virus infection level of bee and brood samples were repeatedly checked throughout the season. A set of closely linked microsatellites allowed to distinguish the genotypes of the 26 drone colonies and to determine the paternity of about 10 worker bees from each of 48 queens mated in three successive periods on the mating station.

The drone population and mite infestation varied considerably between the colonies. The worker bee infestation increased rapidly and had significant influence on the shape of the colonies during the late drone season. The effects of colony development, mite and virus infestation on the realized mating success of individual drone colonies will be presented.

**CHECKING THE EFFICACY OF APIVAR® IN FRENCH COLONIES**

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As Varroa destructor is a major pest of the honey bee (Apis mellifera), its control is a key point in the beekeeping industry. After entering bee larva cells prior capping, V. destructor female lays eggs on the honey bee pupa. Mite nymphs develop in capped brood feeding on haemolymph of its host. In France the use of APIVAR® strips has been the most legally recommended and the most frequently used treatment against V. destructor. However some beekeepers reported that APIVAR® strips were not as effective as they used to be.

An efficacy trial has been run on 15 colonies from our experimental apiary. APIVAR® treatment was applied on ten colonies. Sticky boards were installed at the bottom of the hives in order to collect falling varroas. After 12 weeks, strips were removed and residual mites in colonies were killed using three active ingredients successively: oxalic acid, coumaphos (ASUNTOL®) and fluvalinate (APISTAN® strips). In the treated group, zero to 14 mites were counted during and following these last three treatments. This represents zero to 1.1% of the total number of mites collected during the whole experiment in each of the ten tested colonies. On the opposite, the same three treatments entailed the fall of 233 to 942 mites in the five control colonies to which no APIVAR® strips had been applied.

In our experimental conditions, efficacy of APIVAR® treatment was found to be very high.

Moreover, our results illustrate that a simple checking of mite infestation before winter may give a false security to bee-keepers. A mild number of parasites fallen following a single control does not give account of the long period during which the colony has been bearing a large number of parasites. On the opposite, colonies treated with APIVAR® are freed from most of their parasites since the third week of treatment.
THE EFFECT OF ALTERNATIVE ACARICIDES ON HONEY QUALITY IN ORGANIC BEEKEEPING

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The use of acaricides in organic beekeeping is legally restricted to essential oils and some organic acids, since no synthetic compound can be added to the honey matrix. Thymol, formic and oxalic acid are the main alternatives to control varroa mite, used all over the world with good results. Although these chemicals are already present in honey and should not cause any contamination problem, their continuous use in beekeeping necessarily has some effect in the quality of honey. In this work we followed honey residues of thymol applied, at organic honey production mode apiaries with two different methodologies.

Thymol was applied in paper strips after diluted in olive oil. In each treatment 16 grams of thymol were introduced into a group of eight Langstroth hives. This procedure was repeated during spring (March/April) and autumn (October/November) over three years in four apiaries. Alternatively, thymol was added in impregnated wax foundation with either 9 or 18 grams of thymol per frame. One treatment corresponds to the use of either two frames of beeswax foundations with 9 g/frame or one frame with 18 g/frame. Each one of these latter methods was applied in two groups of seven hives. Honey samples were collected during harvest (September) from all hives, as well as from hives with any varroa treatment. Thymol levels in honey were measured by gas-chromatography with FID detector, after SPE extraction.

From the analytical results we concluded that honey have already a natural content in thymol that seems to increase when thymol is used as acaricide. Although, the levels found do not reach the value of 0,8 mg/kg, (MRL in Switzerland), above it affects the honey taste. Comparatively, the use of impregnated beeswax foundations seems to contribute more significantly to the thymol residues, specially if treated with 18 g/frame.

TOPSY-TURVY BROOD COMBS – IMPACT ON POPULATION DYNAMICS OF HONEY BEES (APIS MELLIFERA L.) AND VARROA DESTRUCTOR

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Since 2003 the Kónya rotating-broodframe beehive (www.anivet.hu) is advertised as a method of augmenting the honey yield, preventing bee colonies from swarming and of Varroa destructor control.

In a triennial study we investigated the effect of unnatural movements of brood combs by 1) weekly rotating all brood combs of 6 colonies during the swarm season, 2) daily manipulating brood combs out of 8 colonies on three different ways: control = comb never touched, rotated = comb rotated 180°, vertically, shaken = comb hit on the ground three times with 40-50g, 3) using the replica of a rotating-broodframe beehive for one season.
Weekly rotation of brood combs neither influenced the population dynamics of honey bees or Varroa-mites nor altered the honey yield. However, in contrast to control colonies most test colonies could be prevented from swarming as weekly rotating causes elimination of swarm cells. Daily rotating or shaking of brood cells did neither affect fertility (93-100%) nor fecundity (2.6-3.0) of reproductive mites or mortality of mite offspring in the brood cells. Independently of the type of manipulation both types of mates were only present in 11-43% of single-infested cells shortly before hatching of the young bee. The mites’ orientation in the brood cell solely seems to be impeded in terms of the position of the fecal accumulation, which was randomly distributed only in rotated cells. A colony kept in the rotating-broodframe beehive died from extremely high Varroa-infestation after one season. Thus, we can not confirm any effect of unnatural movements of brood combs on developing bees or reproductive Varroa-mites, respectively.

CHEMOTACTIC ORIENTATION OF THE SMALL HIVE BEETLE (AETHINA TUMIDA, NITIDULIDAE) IN LABORATORY BIOASSAYS

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The small hive beetle (SHB) is native to Southern Africa where it is a relatively harmless parasite within the honey bee hive. As an invasive species in the USA and Australia the SHB became a threat to beekeeping causing economic damages and even losses of colonies.

One strategy for the treatment and monitoring of the SHB inside the hive is the use of traps in combination with volatile substances which are attractive to the beetle. Therefore, we observed the aggregation and mating behaviour of SHB in plastic cages according to age, mating status and environmental condition. In further olfactometer bioassays we tested the solvent extracts of SHB, which were attractive to other beetles. Additionally, first chemical analysis of extracts from SHB of different age and sex using GC-MS methods are presented.

Under laboratory condition the SHB showed positive reactions toward other beetles of specific stages. However, our preliminary results indicate some obstacles, as we could prove that beetles reared in the laboratory showed differences in their preference behaviour compared to wild beetles, probably due to specific rearing conditions. Further premise for a successful SHB bioassay are revealed through the fact that preference behaviour of the SHB is more pronounced if thigmotactic stimuli are involved in the bioassay.

The requirements for a “standard SHB bioassay” and the possibilities of using the aggregation and mating behaviour of the SHB for a trap within the hive are discussed.

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GENETIC PARAMETERS FOR VARROA TOLERANCE USED FOR ESTIMATING BREEDING VALUES IN THE HONEY BEE

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Breeding value estimation based on a BLUP (Best Linear Unbiased Prediction) animal model is an approved method for the genetic evaluation of traditional traits of the honey bees such as honey yield, gentleness or swarming, cf. the database at www.beebreed.eu.

In order to introduce a more advanced selection criteria which includes the resistance of a bee colony against Varroa destructor an indirect selection strategy is required, because no direct resistance parameters are obviously measurable. Auxiliary traits like the number of dead mites after chemical treatment in summer, the defence behaviour of the bees (proportion of damaged mites) and their hygienic behaviour (guessed by the pin test) which have been collected in Germany during the last years were utilised instead of.

To this end the BLUPF90 code as well as the AIREMLF90 software, which is used to estimate genetic parameters, were adapted to the peculiarities of honey bee pedigrees by introducing a dummy father model in order to approximate the paternal descent at each mating station. Hence the genetic relatedness and inbreeding are estimated more correctly.

Heritabilities estimated by this approach are presented as well as the genetic correlations between the traits. Additionally, an alternative approach for estimating the infestation of the colony by varroa mites is proposed which is based on counting the natural drop of dead mites in spring as well as the number of phoretic mites within a sample of bees taken from the colony in July.

ACARICIDE (FLUVALINATE AND ACRINATHRIN) RESIDUES IN CZECH BEESWAX

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In years 2002-2005 we dealt with analytics and determination of fluvalinate and acrinathrin in beeswax, foundations and propolis by using of gas chromatography and ECD detector by chromatograph Agilent Technologies 6820.

The foundations were obtained from the major providers from Czech Republic. We have not found any detectable amounts of fluvalinate and acrinathrin residues. The detection sensitivity was up to 0,5 mg/kg of wax in fluvalinate and 0,1 mg/kg of wax in acrinathrin. These agents are lipophilous and cannot be eliminated of wax, so they can infiltrate out of wax into honey. The results showed that the residues amount can be ten times lower in honey, i.e. up to 0,05 mg/kg of honey in fluvalinate and up to 0,01 mg/kg of honey in acrinathrin.

The situation in other countries is much worse. Fluvalinate was found in beeswax in amount 1,9-2,9 mg/kg of wax.
The import of cheap beeswax, that could be contaminated with these agents, or the exchange of foreign wax for foundations endanger the Czech honey production at present. The residues contained in this wax could get into the foundations and consequently into honey. In future it is necessary to examine the residual content in foundations.

The results are part of project No. QD1061 from agency NAZV MZe of the Czech Republic.

**SURVIVAL OF HONEY BEES DURING WINTER IN COLONIES INFECTED WITH VARROA DESTRUCTOR**

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When honey bees are infected by Varroa destructor mites during their larval and pupal stage they have a shorter lifespan. In this poster we show how V. destructor infection effects survival of winter bees in an experiment done during the winter of 2005-2006.

**BROOD AND WORKER BEE VIABILITY AND CELLULAR RESPONSES AFTER ACARICIDE APPLICATIONS IN HONEYBEE COLONIES**

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The removed brood from comb cells were counted and marked *in situ* after an oxalic acid (OA)/sucrose or 1% rotenone powder treatment. OA caused 18.7% capped brood removal while 1% rotenone powder 75.2%. The rotenone treatment trig higher brood removal rate (P<0.01) and replacement with young brood than OA or control water treatment. OA solutions of 2.97% OA/31.95% sucrose, 3.40% OA/47.62% sucrose, 3.73% OA/27.10% sucrose and a control, 32% sugar-only solution resulted in death rates of 10.72%, 14.30%, 9.37% and 6.36% respectively in caged worker bees. Bee mortality was higher in the OA treated bees than it was in the sugar solution treated bees (P<0.05). Individually treated bees were prone to drink sugar or OA solutions in negative relation according to the OA concentration. Cell death was detected after OA or formic acids (FA) treatment of honeybee larvae using the TUNEL technique for DNA labelling. In 3- and 5-day-old larvae exposed to OA, cell death was found in 25% of midgut epithelial cells 5 hours after the treatment and increased to 70% by the 21st hour. Fifty hours after the application, cell death was reduced on to 18% of the epithelial cells of the 3-day-old larvae and had increased to 82 % in the 5-day-old larvae. Evaporated FA induced extensive apoptotic cell death in the peripheral, cuticular and subcuticular tissues that preceded the cell death of the entire larval body. In old worker bees cell death was detected in 48% of the columnar epithelial cells of the midgut 12 hours after an OA treatment, falling away to 20% after 24 hours. The possible effects of OA on the tissues of brood stage or adult worker bees are discussed in this study.
COURSE OF VARROA DESTRUCTOR INVASION IN BEE COLONIES TREATED WITH AMITRAZ

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In 1987-2005 in 20-30-hive stationary apiary, bee colonies were treated against Varroa destructor with preparation containing 12.5 mg of amitraz (Apiwarol AS – in the form of a fumigating tablets, Biowet, Poland) according to the own programme; additionally sealed drone brood was cut off. The efficacy of Varroa control was evaluated after the end of treatment (September).

In 1987-1991 females of V. destructor were found in all bee colonies, although its number on bees varied and fluctuated between 0.6 and 4.5 in sample of 100 insects.

Since 1992 number of colonies, in which parasites were found after the last treatment, decreased to 19-52% as well as number of Varroa mites in 100 bees (to 0.4-0.7).

The results of presented studies indicate, that regular applying of amitraz by spring 1991 and next after the last honey harvest, and since 1992 only late-summer fumigation (end of July-September) in visible way reduced extensity as well as intensity of V. destructor invasion.

This so low intensity of V. destructor invasion in bee colonies shows high effectiveness of amitraz and lack of parasite resistance to this substance. The presented manner of varrosis treatment combined with cutting off sealed drone brood, turned out very effective and cheap.
Honey bee genetics

Symposium organized by Michael Lattorf

A THIRD GENERATION LINKAGE MAP OF THE HONEY BEE, APIS MELLIFERA

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The construction of the genetic map of the honey bee genome was initiated ten years ago and was constructed using the genotypes of worker progeny of one or two queens (respectively 92 and 95 workers per queen). It involved three steps:

The first version was mainly built with microsatellites prepared at the laboratory (screening of genomic libraries and sequencing of positive clones, Solignac et al., 2003) and was recently published (Solignac et al., 2004). This map comprised 541 markers on 24 linkage groups and hence was not saturated, the complement comprising 16 chromosomes.

The second version of the map was considerably denser thanks to two sources of sequences that became available in Genbank. The first source was a library of about 15,000 sequences of cDNA prepared from brain (Whitfield et al., 2002). A priori such sequences are not highly favourable for microsatellite extraction but we were able to map 493 markers prepared from these expressed sequences. In addition, a first read of short sequences of the genome also became available and 108 markers were prepared from them. A total of 601 markers was thus added which allowed saturating the map. This second generation was not published but was used for the first assembly of the genome sequences.

From this time, the successive assemblies became an inexhaustible source of new markers. Mapping took advantage of the sequence to considerably increase the density of the map. On the other hand, the map was useful for the sequence to assign the scaffolds to their respective linkage groups, to order them within these groups and to add numerous previously unplaced scaffolds to their respective chromosomes. Moreover, interaction between the two approaches also allowed eliminating most of mapping and assembly errors.

The genetic map of the honey bee is now based on 2,008 microsatellite markers with a total genome length of 4,114.5 cM (Kosambi function of distance). The genetic length of linkage groups varies from 575.9 to 138.0 cM The density of markers is 2.05 cM (corresponding to a physical length of only 93 kb and 5 to 6 genes) and all genetic distances are lower than 10 cM.

Analysis of the queen meiosis reveals that (i) recombiantion rate is a linear function of the physical distance and is homogeneous for all chromosomes and (ii) chiasmas are subjected to positive interference suggesting a highly regulated process.
SELECTION OF HONEY BEE STRAINS FOR POLLINATION OF SPECIFIC CROPS

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The value of increased agricultural production attributable to honey bee pollination is much higher than the direct value of hive products. Still, most of the selection and breeding effort is aimed for increased honey production and better resistance to bee diseases and pests and not for better pollination performance. One of the main problems in using honey bees for pollination is that bees placed in the target crop often neglect it in favor of competing flora. We studied the genetic background of bee preference for two important bee-pollinated crops that suffer from competition. In apple, pollen collectors are the main pollinators. We found significant differences between various genetic strains in the proportion of apple pollen that they collected. Furthermore, colonies that were progenies of colonies with high preference for apple pollen one year ('High strain') tended to collect a higher proportion of apple pollen than 'Low strain' colonies the following year.

In avocado, nectar collectors are the main pollinators. Based on the amount of the unique avocado sugar perseitol in their honey, we estimated that bees of the New World Carniolan strain collected 1.4 to 18.1 times more avocado nectar than Italian bees in the northern part of Israel. This trend repeated for several years, but was confounded by a gene-environment interaction. A genetic basis for the propensity to visit avocado flowers was further supported by the consistently high honey perseitol content of selected colonies over two years. A genetic component for preference to target crops attests the possibility of breeding honey bee strains that will be effective pollinators where competition with alternative flora may be a problem.

DISPERSAL DISTANCES AND DRIFTING OF DRONES

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Mating of honeybee drones and queens (Apis mellifera) takes place in midair at drone-congregation-areas (DCAs). It is unknown, how far drones are actually able to fly and whether they return to their mother apiary or return to another apiary after a mating flight. Both aspects may influence the management of populations but also the control of parasites and diseases. To examine the mating flight distances of drones, we combined a capture-mark-recapture experiment with genetic parentage analyses. Drones were captured and marked at a DCA in a study area (radius: 2.5 km) where all occurring queens were genetically known. Of all marked drones 47% were recaptured in the hives within the study area. The genotype of each queen was compared with the ones of the recaptured drones using 12 microsatellite loci. We found that a majority of drones drifted within their apiary of origin but no drifting among the apiaries within the study area could be detected. Nevertheless 3% of all recaptured drones did not descend from any known queen within the study area and were therefore identified as dispersers. Neither the homecoming drones nor the drifting drones were equally distributed among the hives.
within an apiary. Our results suggest that drones at a DCA are not a random sample of the population and that the number of drones contributed by apiaries or hives may not follow a simple distance-dependent relationship. The results on long-distance dispersal of male honeybees are discussed in regard to the conservation of endangered subspecies and their potential role as a vector for the spread of diseases.

GENETIC STRUCTURE OF TURKISH HONEYBEE POPULATIONS BASED ON RAPD AND mtDNA RFLP MARKERS

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The aim of this research is to determine the genetic structure of Turkish honeybee populations based on RAPD and mtDNA RFLP markers. 175 honeybee colonies were sampled from 16 different locations. A total of 149 amplified bands were scored from 20 RAPD primers and 92.6% (138 bands) polymorphic bands were found. Based on RAPD markers the average heterozygosity (H) was 0.331. High genetic differentiation was found among subpopulations with genetic distances (D = 0.0716-0.2283) and the average coefficient of population differentiation (\( R_{ST} = 0.2889 \)). The average coefficient of population differentiation revealed that 71.11% of total genetic diversity (\( K_T = 0.3299 \)) was within subpopulations (\( K_S = 0.2346 \)). On the other hand gene flow (\( N_{mN} = 1.2301 \)) was very low. The mtDNA of honeybees were characterized by DraI restriction profile of the COI–COII intergenic region, HincII and HinfI restriction profile of cytochrome oxidase I (COI) gene, BglII restriction profile of cytochrome oxidase b (cytob) gene, EcoRI restriction profile of large subunit of ribosomal RNA (lsrRNA) and Xbal restriction profile of inter COI–COII region within the COI gene. BglII digestion in Cytob gene, EcoRI digestion in lsrRNA and XbaI digestion in inter COI–COII region within the COI gene was present in Turkish honeybees. On the other hand HincII and HinfI digestions were absent in Turkish honeybees. In the COI-COII intergenic region, DraI digestion revealed 3 restrictions that gave 420, 64, 49 and 41 bp. fragment size that may be a new haplotype in the C1 pattern of the Mediterranean lineage.

MICROSATELLITE REPEATS IN THE HONEYBEE GENOME

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The honeybee, Apis mellifera, is the third species, which genome has been sequenced completely. Besides eusociality honeybees differ from other species with respect to their genetic system, which is characterized by male haploidy, and a complementary sex determination system. The genome has the highest recombination rate amongst multicellular eukaryotes. These special genomic features seem not to affect tandem repeat characteristics like nucleotide content of DNA, motif lengths and
frequency distributions of tandem repeats. For 19 eukaryotic genomes the frequency distributions of tandem repeats showed phylogenetic footprints indicating selective processes rather than random evolutionary change. Nevertheless, the distribution of motif types for di-, tri- and tetra nucleotide repeats also show phylogenetic footprints. The only exception is the honeybee, which shows a completely different composition of microsatellite motifs. Moreover, there is a highly significant correlation between recombination rate and number of tandem repeats in 19 eukaryotic genomes. The honeybee significantly deviates from this pattern in that it has more repeats then expected due to its high recombination rate.

SEASONAL VARIATION OF PHOSPHOGLUCOMUTASE (PGM) ENZYME POLYMORPHISM IN HONEYBEES (APIS MELLIFERA L.) OF TURKEY

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The allozyme variability on many loci of several species is associated with a variety of morphological, physiological, and fitness-related traits. Climatic conditions also can affect the physiological performance of organisms through their influences on enzyme function.

Phosphoglucomutase (Pgm; 2.7.5.1) is an enzyme that contributes to the regulation of energy metabolism by catalyzing the conversion of glucose-1-phosphate to glucose-6-phosphate at glycolitic pathway.

In a previous study on honey bees Pgm was found as the most polymorphic locus with three alleles (F=fast, M=medium, S=slow) and it exhibited polymorphism in all of the provinces in Turkey. Applying horizontal starch-gel electrophoresis we had determined the Pgm genotypes of worker bees that were collected from the same hives belonging to three apiaries, in each month from October to April, 2006 for the time being, that will continue to cover a whole year. Based on seven month collection of 140 worker bees, the level of heterozygosity we detected is quite remarkable; heterozygote (MF) frequency had declined from 1.00 to 0.80 and the frequency of homozygote, (MM) increased from October to April. The frequencies of F, M, and S alleles were 0.486, 0.511, and 0.004 respectively.

Characterization of thermostability variants and measurements of enzyme activity parameters for different Pgm genotypes will allow us to relate biochemical characteristics to changes in enzyme genotype frequencies. Investigation of mechanisms by which honeybees respond to temperature changes will contribute to the understanding of biochemical and physiological consequences of the Pgm polymorphism and seasonal variation of its genotypes.
BREEDERS SELECTION WORK IN SERBIAN APICULTURE

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There are around 20,000 apiculturists in Serbia nowadays who own around 320,000 bee hives. The possibilities of Serbia for bee breeding are much greater than the present number of bee colonies and it is estimated that it can be as much as 800,000 bee hives. Based on several years of research and surveys of apiculturals it is estimated that the annual need for selected queen bees in Serbia ranges between 50,000 and 60,000. With regard to the great need for quality biological material five centers for the selection with breeded material have been established, and a network of reproductive stations is under elaboration.

Breeders selection work is based on selecting and protecting ecotypes Apis mellifera carnica in which 39 morphological parameters and several important productive features are investigated.

Based on morphometric researches three lines of bees, on the sample of three lines with 30 bees each, the length of tongue varied from 6,2264 to 6,4397 mm and it was statistically significant, while the measures of cubital index ranged from 2,55 to 2,89 and were statistically very significant. The number of hooks on the rear wing was not significantly different in the tested lines and ranged approximately from 21.49 to 21.85. The length of the front wing ranged from 8,8771 to 8,9168 mm with very low variation coefficient (0,86 % and 1,74%), while a statistically significant difference was determined by measuring the length of the rear wing (variations from 6,2378 to 6,3585 mm).

By researching honey productivity of some selection lines it was established that the lines of the central region (21, 78 and 27,13 kg) had the greatest yield of honey per bee hive in 2004 and 2005. Studying the behaviour of bees with pin test we determined the difference of 14.25% after 24 h, i.e. 15.05% after 48 h between certain lines.

After the first study phase which included the investigation of morphometric and productive economic characteristics, genetical DNA analysis of is planned which is expected to be in accordance with the established differences in the previous research phase.

THE MALE GENITAL SYSTEM OF APIS MELLIFERA: A MORPHOLOGICAL APPROACH

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In spite of their rather short lives, males perform an extremely important function, as it is only after insemination with their sperm that queens are able to produce female offspring. Copulation occurs high in the air on the wing. Sperm transfer is a complicated process during which the male everts and injects his endophallus into the queen. The endophallus can be divided into three parts, the vestibulum with the paired cornua, the cervix, and the bulbus accompanied by the lobe. The most conspicuous part of the...
endophallus, the bulbus, is very complex. Almost nothing is known concerning its functioning. We therefore investigated its detailed structure. In order to get more insight, we started an ontogenetic study, focusing on the pupal stage of the drone. It starts as an elongated tube which progressively invaginates and curves. For adult drones, we describe the different epithelial regions of the bulbus “gland”, including its externally located epithelia and its more internally located epithelia. The dorsal epithelium above the chitine plates appears to be secretory active and is highly cylindrical prior to the sexually mature stage. Its cuticle encloses greenish droplets within an extremely irregular cuticular matrix. This is in contrast with the ventral epithelial lining which is highly squamous. The ventral and dorsal epithelia differ from the anterior and the posterior epithelia. Besides the bulbus, we also examined the mucus glands. These glands have three parts, the muscle layer, the glandular epithelium and the lumen which change with respect to age in the distal and proximal region.

THE WEIGHT CHANGES AND REPRODUCTIVE TRAITS OF HONEY BEE QUEENS (A. M. CAUCASICA)

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Weight changes of honeybee queens reared from a breeder caucasian colony (A. m. caucasica) were monitored during reproductive development after emergence. The mature queen cups kept in an incubator were inspected at 5-min intervals and emerging queens were boxed immediately after weighing. The queens in mating boxes were inspected regularly to identify the days when they mated and started egg laying. The queens were weighed at different periods and when they became 1-month old, they were dissected.

Five of 50 queens (10%) were lost during pre-mating period and 2 of 45 queens (4.4%) did not return from mating flight. Twenty one of 43 queens (48.9%) mated once, while 22 queens flew out second time. All mating flights were performed in the late afternoon between 16.00-17.30. The average interval from emergence to first mating flight and the average onset of oviposition after emergence were 6.9 days and 10.6 days, respectively. The mean weight of queens at emergence, 3 days after emergence, one day after last mating flight, the day of onset of oviposition, three days after onset of oviposition, and 1-month old were 195.9 mg, 164.8 mg, 159.6 mg, 203.6 mg, 220.9 mg, 214.1 mg, respectively. The weight of queens at emergence decreased continuously till mating. After mating they gained weight. The diameter and the volume of spermatheca, the fresh and dry weights of ovaries, and the number of spermatozoa entering the spermatheca were found to be 1.1 mm, 0.7 mm³, 53.7 mg, 9.4 mg, 4.877.000, respectively. There were significant correlations (P < 0.01) between the weight at emergence and the diameter of spermatheca (r = 0.619) and the volume of spermatheca (r = 0.607).
STUDY ON FACTORS ACCELERATING OVIPosition OF INSTRUMENTALLY INSEMINATED QUEEN BEEs

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Investigations on the improvement of instrumental inseminations of queen bees are conducted in Poland from the beginning of the 60ties in XXth century. The precursor of these investigations is prof. J. Woyke. One of the results is the common use of instrumentally inseminated queens in most apiaries in Poland. This speed up the breeding progress of bees, and results in increase of honey production by the apiaries.

Nowadays, Poland is the largest producer of instrumentally inseminated queen bees in the world. We are producing some thousands of instrumentally inseminated queens each year.

Besides unquestionable advantages, instrumental insemination has some disadvantages. The greatest obstacle is the prolonged period of starting oviposition by instrumentally inseminated queens, which may last from 4 days to a dozen or so. Beekeepers maybe discouraged to buy instrumentally inseminated queens, which start egg laying after many days, because lack of brood for some period results in decrease of honey production. It should be however, noticed that the breeding value of queens starting oviposition later is the same as of those beginning egg laying earlier.

Therefore three Bee Divisions of Agricultural Academies in Olsztyn, Szczecin and Warsaw, Poland, undertook investigations on accelerating the start of oviposition of instrumentally inseminated queens.

Experiments were carried out from 2003 to 2005, three times in each season. Carniolan bees (Apis mellifera carnica) were used in this investigation. The queens were introduced into trapezoid mating nuclei. The nuclei with accepted queens were randomly divided into 6 groups, of 15 nuclei each.

All queens were inseminated with 8 cu mm of semen at the age 8 day. They were treated with CO₂ for 3 minutes, once at the age of 6 days and the second time during insemination.

Plugging with mucous and flying were chosen as possible equivalent of natural factors, like mating sign and mating flight of queen bees.

INFLUENCE OF VARIOUS CONCENTRATIONS OF CO₂ ON RESULTS OF INSTRUMENTAL INSEMINATION OF HONEYBEES QUEENS

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Carbon dioxide is the most often used anaesthetic during insemination. Negative effects are the more harmful the longer exposure to anaesthetic and the older individual is narcotized. Ebadi and Gary (1980) used to bee queens’ narcosis a various CO₂ concentrations by mixing pure CO₂ with air. Bee queens narcotized with diluted CO₂
started oviposition earlier than those treated with pure CO₂. The objects of the study were to determine the optimal dose and CO₂ concentration and its effects on honeybee queens. The study was run in the laboratory of Bee Breeding of the Institute of Pomology and Floriculture, Apiculture Division in Pulawy in 2004, 2005 and 2006. The sister Carniolan queens after emerging were placed in mating hives. Queens were instrumentally inseminated when 8 days old with 8 µl of semen of Caucasian drones using 50%, 75%, 80%, 90% and 100% carbon dioxide. The time to immobilize the queen, the time to wake up the queen and latency period were estimated. A control group was naturally mated queens.

Queens treated with 100%, 90%, and 80% CO₂ concentrations were immobilized faster, respectively in 13.8, 20.3, and 23.2 s. Queens treated with 75% CO₂ were immobilized in 33.8 s and with 50% CO₂ in average 99.1 s. The shortest walking up time was noted in group of queens treated with 50% CO₂ and it was 19.9 s. from the moment of insemination. Out of all examined queens, 87% started oviposition average in 16.6 days after insemination. The shortest latency period was observed in group of queens treated with pure CO₂ (10.4 days) and significant longer in other compared groups (21 to 27 days). There were significant differences between means for years of research.

INJURIES FORMATION IN PRODUCTION OF ARTIFICIALLY INSEMINATED QUEENS AND ITS INFLUENCE ON THEIR USEFUL VALUE

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Observation of injuries of artificially inseminated bee queens during all period of their production was done. The effect of bee queens injuries on their useful value and workers behaviour was examined. Queens before insemination were kept with attendant bees in cages in room temperature, and after the insemination they stayed in incubators. Before introducing queens into commercial colonies they were introduced to nucleuses to time of oviposition. The period of production of instrumental inseminated queens has been divided into 4 steps related with queen age and production technology: I-from emerging to insemination, II-from insemination to 2nd CO₂ treatment, III-from 2nd CO₂ treatment to acceptation in nucleuses, IV-from introducing to acceptation in commercial colonies. The criteria of elimination of those stages was also a contact with new workers which could cause queen injuries. Queens with similar injuries were divided into following groups: A-uninjured-as a control group, B-injured arrolium and claws, C-missing legs or its part, D-paralyzed legs, E-injured antennae, F-injured wings. The greatest number of queens (83 %) had one leg injured with no preferences to the pair of legs. The older queens the greater number of injured individuals. In the stage I- 2 %, II-4 %, III- 13 % and IV- 32 %. Also losses of queens were greater in following stages and differences were significant. Queens body weight has no influence on its injuries and losses. Workers in commercial colonies accepted queens with injuries and uninjured ones. Brood rearing in bee colonies with queens from B and C groups was similar like in colonies with uninjured queens. Substantially lower number of brood was in colonies with queens from groups D and E. Injured queens were more frequently superseded by bees than uninjured ones.
Diversity and conservation

Symposium organized by Maria Bouga and Lionel Garnery

QUEEN MATING FREQUENCY IN POPULATIONS OF APIs MELLIFERa IBERIENSIS BELONGING TO DIFFERENT EVOLUTIONARY LINEAGES

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The mating frequency of two unmanaged populations of Apis mellifera iberiensis, one located at the north of the Iberian Peninsula corresponding to the mitochondrial West-European (M) evolutionary lineage, and other one at the southeast bearing African (A) mitochondrial DNA, has been estimated using five microsatellite loci. A total of 1441 workers were genotyped and the estimated, effective and observed numbers of matings were calculated from the workers offspring. The queens of the twenty-two M colonies analysed mated 8-21 times with an average relatedness among workers of 0.303±0.020. The observed number of matings per queen in the twelve A colonies ranged from 12 to 25 and the genetic relatedness among these workers was 0.294±0.102. These results are within the range of those found in other Apis mellifera subspecies. A significant difference (p=0.0548) has been detected between the two types of honeybee populations in relation to the observed number of matings (15.7±4.6 averaged for those colonies corresponding to the M lineage and 18.9±6.5 for the colonies belonging to the African lineage). These results suggest that ecological factors as the location of the colonies and climate conditions as well as evolutionary trends may influence the queen’s mating behaviour.

INTROGRESSIONS AND STRUCTURE OF THE GENETIC DIVERSITY OF THE BLACK HONEYBEE IN FRANCE

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The objective of this study is to assess the situation of the black honeybee in France. A large survey has been conducted over the past three years (2003-2006) to determine the levels of introgressions and genetic diversity of honeybees in various regions of France and in South of Belgium. A total of about 3800 colonies were sampled and analysed (mtDNA for all colonies and microsatellites for a few populations). Fifty-one new haplotypes were discovered in France, doubling the total amount of haplotypes known in the M lineage. These results highlight the high diversity found in populations of the black honeybee. This diversity is geographically highly structured. At a large scale, it exhibits a sharp gradient from south-west to North-East of France with predominance of haplotypes M4 and M19 in the south and M17 in the north. At a finer
scale, local differentiations appear that need further investigation to determine if they are associated to local differenciations. Further discussion is given on the structure of the diversity found in France compared to other European countries where A. m. mellifera naturally occurs.

**DISCRIMINATION BETWEEN HONEYBEE SUBSPECIES BASED ON GEOMETRIC MORPHOMETRICS**

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Discrimination between honeybee (Apis mellifera) subspecies is usually performed by methods of traditional morphometrics, which is based on multivariate analysis of distances, angles and ratios. Those methods proved to be successful, however, there are some difficulties related to interpretation and graphical presentation of results. Many of the problems of traditional morphometrics were solved by geometric morphometric. The new method is based on landmarks, which are described by Cartesian coordinates.

The methods of geometric morphometrics were used to analyse differences between three honeybee subspecies: A. m. mellifera, A. m. carnica and A. m. caucasica. From every subspecies a hundred workers were collected and their forewings were dissected. As landmarks 18 vein junctions were chosen. After obtaining the wings images the vein junctions were detected automatically using DrawWing software. Generalized Procrustes analysis followed by Canonical Variates Analysis was used to compare the shape of venation. The discrimination based on the geometric morphometrics proved to be successful. The analysis revealed many differences between the subspecies, which are difficult to detect using other methods.

**MORPHOMETRIC ANALYSIS OF HONEYBEE IN NORTHERN TURKEY**

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The goal of this study is to determine the variation of honey bee populations and also whether migratory beekeeping or importation of commercially produced queens would influence the natural distribution.

Honeybee samples were collected during May-August of 2002-2005 in northern Turkey, including South Marmara and Thrace along the coast from the Georgian to the Bulgarian border. A total of 58 colonies were sampled ranging from sea level up to 3000 m. Bee samples were taken from remote villages with no or minor migratory beekeeping activities within short distances and preserved in 70% ETOH until analysis. 38 morphometric characters (Ruttner 1988) were measured by using a stereo microscope and a PC-based video measuring system (Meixner 1994). The data were analyzed with
the SPSS using factor analysis and discriminant analysis. Reference samples from the Oberursel data bank were included in the analysis. The results of the morphometric analysis suggest that A.m. anatoliaca is the predominant race in Northern Turkey extending into Thrace region. A. m. caucasica is prevalent only in some Eastern Black Sea locations. Even though the bee samples were taken from stationary beekeepers and native racial distribution is predominantly preserved, some mixing was apparent throughout the region.

GENETIC DIVERSITY OF BEE ECOTYPES IN TURKEY-GREECE BORDER LINE USING MORPHOMETRIC AND MOLECULAR MARKERS

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According Ruttner’s morphometrics analysis (1988) A.m. syriaca, A.m. meda, A.m. anatoliaca and A.m. caucasica occur in Turkey, as well as, A.m. macedonica, A.m. adami, A.m. cecropia and A.m.carnica in Greece. In this study honey bees from 36 colonies from 4 different areas of Turkey-Greece border line were surveyed with morphometrics analysis using twelve characters and molecular analysis using PCR-RFLP’s method, on two mtDNA gene segments. The results of the morphometrics analysis were statistically processed using NTSYS program package; as concerning the results from mtDNA analysis REAP and PHYLIP packages were being applied.

The results of our research from both approaches show that populations studied are not the same. Diagnostic patterns have been revealed discriminating Greek and Turkish honey bees concerning mtDNA analysis. The above mentioned results could be useful for conservation purposes concerning local honey bee populations.

NATIONAL CERTIFICATION SYSTEM OF HONEY BEE QUEENS IN GREECE

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Since August 2005, the Laboratory of Agricultural Zoology and Entomology of AUA and the Hellenic Institute of Apiculture of NAGREF, have started an attempt to establish the National Certification System of Honey bee Queens in Greece. According to the above system, the Hellenic Accreditation System following the procedures of ELOT EN 45011 will accredit two bodies for Certification. The two bodies involved will be independently accredited, but they will collaborate very closely, also with other accredited laboratories. The Laboratory of Agricultural Zoology and Entomology will handle all procedures concerning the genetic origin of the queens produced in Greece and the Hellenic Institute of Apiculture will measure several parameters affecting the
quality of the queens. The above system is co-financed by the EU and the Greek Government for 2006 and 2007.

APIS MELLIFERA SICULA MONTAGANO POLYMORPHISM INVESTIGATION BY MOLECULAR AND BIOCHEMICAL METHODS

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The genus Apis includes species mainly located in Asia. The unique species located in Africa, Europe and western Asia is Apis mellifera L.. In Italy this species includes 2 subspecies A.m.ligustica Spinola and A. m. sicula Montagano. This strain is present essentially in Sicily and because of less cross breeding selection original wild characters are probably well preserved. Biochemical characterization is classically used as tool to identify the different strains. However to aim the biodiversity preservation, in the last few years several studies had been started to identify molecular markers that can distinguish among different subspecies. Molecular approach can be useful to improve the classification systems and to discover more informative polymorphic sites. In this paper preliminary data on genetic polymorphism among the two different subspecies A. m. ligustica and A.m.sicula by analysis with different molecular approach (restriction analysis in mitochondrial DNA, AFLP, microsatellites), identified by iso-enzymatic pattern, are reported.

HYBRIDIZATION AMONG EUROPEAN SUBSPECIES OF THE WESTERN HONEYBEE APIS MELLIFERA

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Modern Beekeeping practice has lead to many introduction of European honeybees into formerly bee free areas and areas preoccupied by other subspecies. The sudden sympatric occurrence has lead to the hybridization of subspecies of the Western honeybee Apis mellifera and population amalgamation would be expected to be the long term results. Especially subspecies with no or very few remaining allopatric populations are feared to be highly threatened by hybridization. The example of the Africanized honeybee has shown that complete admixture is not necessarily occurring if different honeybee subspecies are mingled. Many scenarios have been proposed in order to explain the unidirectional and non-random introgression proportions among European and African subspecies. Among European subspecies, many studies have shown, that hybridization does occur, but so far it is unknown to what extend hybridization leads to genetic introgression. In this study we quantify genetic differentiation and admixture proportions between the endangered A.m.mellifera and south-eastern European subspecies in populations with different levels of artificial selection. We show that there still is a high level of differentiation among the different subspecies. Although fertile hybrids are produced, admixture patterns show that there is a barrier to hybridization that prevents subspecies from complete admixture. This barrier can not be explained by
artificial selection alone, what leaves the implication, that there exists a mechanism that hinders hybridization to some extent.

**MICROSATELLITE ANALYSIS REVEALED THE MAINTENANCE OF GENETIC IDENTITY OF SUBSPECIES OF APIS MELLIFERA L. IN TURKEY IN SPITE OF INTENSIVE MIGRATORY BEEKEEPING**

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Despite the intense migratory beekeeping within the last three decades, a very high allozyme diversity have been observed between the honeybee populations distributed throughout Turkey. Our aim was to assess the present genetic structuring in the honeybee populations of Turkey using nine microsatellite loci, since it is claimed that gene pools belonging to different subspecies lost their integrity because of hybridization due to intensive migratory beekeeping. Honeybee populations were sampled from 11 provinces in Turkey and an additional sampling was carried out from Cyprus.

We have observed great genetic diversity among populations as indicated by significant pairwise FST values for the majority of population pairs, 52 out of 66 pairs. Also the most of the Nm values (pairwise number of migrants) were lower than 2 which indicates significant reduction of gene flow between these populations. The percentages of correct assignments of individuals to the population they were sampled, revealed a highest score as 87% and a 62% mean correct assignment score with a great variance because of the 3 populations that had very low assignment percentages. These populations were on the major routes of migratory beekeepers. The strategies for conservation of genetic diversity of well-adapted local honeybee populations in Turkey are being developed.

**CONSERVATION AND INCREASE THE NATIVE POLLINATOR NUMBERS IN NATURAL HABITAT**

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The essence of ecologization of entomophilic crop cultivation is to bring insect-pollinated crop ecosystem function near to natural ecosystem function, i.e. to imitate natural processes, based on a balance basis, conservation and reproduction of wild life resources (Dobrynin, 1998, 2004). Native wild bee species are classified as valuable natural resources as well. Accordingly, the solution of the problem of sufficient supply of entomophilic crops with adequate pollinators should be based first on the active utilization of native widely spread bee species. There was developed the system of measures on conservation and increase the native pollinator numbers in natural habitat ((Dobrynin, 1998).

This system consists of the following components:
1. Conservation and creation of native bee nesting habitats which include: protection of existing (creation of microrefuges) and enlargement of natural nesting habitats; establishment of new habitats favorable for pollinators nesting (imitation of natural nesting habitat); ecologization of techniques of establishment and management of entomophilic crop agroecosystems.

2. Enlargement and management of forage resources of native bees which include: imitation of natural change of flowering vegetation in limits of agroecosystems (using of bait crops, flowering adjustment and rational utilization); enlargement of entomophilic crop agroecosystems.

3. Pollinator protection in a system of chemical crop protection taking into consideration: ecological features of pesticide-pollinator relationships (the role of abiotic and biotic factors in pesticide–pollinator relationships, influence of pesticide properties, regulations and objects of application on pollinators); special features of entomophilic crop protection; protection of pollinators during pesticide application.

FLOWERING SET-ASIDES IN LARGE CULTIVATED ZONES: CONVERGING INTERESTS FOR FARMERS, THE BIODIVERSITY, LANDSCAPES AND BEEKEEPING

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Recently a decline in the bee populations has been observed in Europe. This assessment raises the question of the possible risk of the disappearance of all pollinating insects and the repercussions on human activities based on the survival of these insects (beekeeping, fruit, vegetable, and seed production). A reason for this decline will be the destruction and the fragmentation of the bees’ habitat. The preservation of their habitat is necessary but has never been taken into account. Fallowing area initiated by the European common agricultural policy might be an opportunity for apiculture and more generally for insect pollinators conservation. Appropriately sown, these agricultural surfaces could offer various sources of nectar and pollen. Agro-environmental set-asides might have positive impacts on insect populations by giving them habitat and food. European regulations impose fallowing area to be spontaneously or artificially sown. Allowed species are recorded in a list which can be modified on a regional scale. In France, specific clauses have been generated from the general frame in order to respond to particular situations: set-asides intended to preserve biodiversity and protect water quality. Evolution of rules and regulations as well as agricultural practices is necessary in order to improve insect population development. Sowing fallowing area with melliferous and polleniferous species would be a good contribution to promote beekeeping and protect biodiversity.
DETERMINATION OF GENETIC VARIATION IN NORTHERN IRAN HONEYBEE (APIS MELLIFERA MEDA) POPULATIONS USING MICROSATELLITE AND RAPD MARKERS

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The main objective of the present study was to analyze the genetic structure of four honeybee populations (Sarein, Amol, Urmiyeye and Viladereg) from northern Iran using five microsatellite loci and RAPD primers. Average heterozygosity was estimated between 0.583 and 0.730 based on microsatellite data. Heterozygosity values for Iranian populations were found higher than European populations (0.230-0.395) but lower than the tropical African populations (0.756-0.896). Genetic differentiation among populations was estimated by using fixation index (FST) ranging from 0.032 to 0.142. Linkage disequilibrium tests for all pairs of loci at all populations detected 8 significant values between pairs of loci. The gene flow (Nm) between Iran populations was estimated as 1.570. Hence there is significant genetic differentiation among populations. A phylogenetic tree was constructed based on genetic distances among populations and clustered the Urmia-Viladereg into one and Amol-Sarein into another cluster.

On the other hand, genotype assignment tests based on the RAPD data analyzed by AFLPOP which does the assignment of the likelihoods of each individual for different population pairs revealed distinction between Amol-Viladereg and Sarein-Viladereg population pairs.

MOLECULAR PHYLOGENY OF A.MELLIFERA SUBSPECIES OCCURRING IN GREECE BASED ON SEQUENCING OF TWO MTDNA SEGMENTS

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Sequence data from two mt DNA segments of honey bee populations from different regions of Greece: Evros, Chalkidiki, Kefalonia, Rhodes, Crete, Macedonia, Messinia, Limnos, Nisyros, as well as from Cyprus, Italy and Albania, were used to investigate their phylogenetic relationships.

The mtDNA segments analyzed was a portion of COI and ND5, amplified using PCR after the extraction of total DNA. A total of 910bp and 688bp respectively were obtained by sequencing the above mentioned segments. CLUSTAL package was used for the alignment of these sequences. Nucleotide divergence was calculated using MEGA package and cladogram was constructed with various phylogenetic packages.

The results of our investigation show that no considerable interpopulation variability was detected.
BIOMONITORING IN AN ITALIAN NATURAL RESERVE

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A monitoring study in a natural reserve of Northern Italy (Regional Park of Colli Euganei) was set up in 2003 and 2004 with the aim to test environment quality using honeybees as biological indicator. In particular, agricultural pollution was determined by the analysis of dead bees, whereas the presence of heavy metals in honey was used to verify air quality. The monitoring station was composed of two beehives of similar strength and equipped with “underbasket cage” to collect dead bees. Beehives were weekly monitored for strength, development, bee mortality and diseases. Dead honeybees and samples of honey were be collected, properly stored and analysed for agricultural pesticides and heavy metals (cadmium and lead) residues.

During 2003 five stations were recruited and distributed in rural areas characterised by orchards, olive-groves, vineyard and sown lands. The programme started in April and continued until September. The honeybees mortality never reached the critical threshold and the pesticides analysis resulted negative. The lead content in honey ranged from 110 and 160 ng/g, whereas the cadmium mean level was 1,5 ± 1,7 ng/g.

In 2004 nine monitoring stations were used from June to November and located either in urban or rural areas. The honeybees mortality was < 50 bee/hive/week and the pesticides analysis of dead bees resulted negative. Honey samples were taken either from super or nest. In the first case lead and cadmium mean content was 192,6 ± 118,5 ng/g and 5,2 ± 2,5 ng/g respectively, whereas in the nest honey lead residues ranged from 214 and 488 ng/g and those of cadmium from 4 e 14 ng/g. Weather conditions (poor rainfall) and stations located close to urban areas could explain the higher heavy metals content in samples collected in 2004 than 2003.

VARIATION OF MITOCHONDRIAL DNA IN HONEY BEES OF JORDAN

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The honey bee of the eastern Mediterranean, A. mellifera syriaca, is found in the regions of Syria, Lebanon, Israel and Jordan and occupies an interesting place in the biogeography of the species as the most south-western of the oriental morphological already neighboring African bees. Its genetic integrity is endangered by persistent honey bee imports of commercial breeder lines into the region. A total of 25 samples of honey bees were collected from 14 locations throughout Jordan. Total nucleic acids of one individual worker per sample were extracted with a modified phenol-chloroform method (Arias and Sheppard 1996). A mitochondrial fragment containing the intergenic region between the tRNAleu gene and the second subunit of the cytochrome oxidase gene was amplified using the primer pair E2-H2 (Garnery et al., 1993) and digested with the restriction enzyme DraI. Restriction fragments were separated on 10% polyacrylamide gels, stained with ethidium bromide and photographed under UV illumination.
Of the 23 samples that gave positive amplifications, seven were characterized by haplotypes of the C lineage and 16 by haplotypes of the mitochondrial O lineage as described by Franck et al (2000). Haplotypes C1 and C2 were predominantly observed in the north of the country, whereas haplotypes of the O lineage were distributed throughout Jordan. One new haplotype (O3’) was found. While the presence of the C2 haplotype can be explained with natural admixture between lineages C and O in Jordan, the occurrence of the C1 haplotype strongly suggests past or recent importation of A. m.ligustica queens. Haplotypes were mostly consistent with morphometric allocation of bees to the subspecies.

**EUROPEAN BEES, THEIR MORPHOLOGY AND MICROSATELLITES**

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The protection and use of bees’ gene pool in special programs, in rearing and in beekeeping practice cannot omit the description of the belonging to races. The description of gene types were afore based only on pedigree records and phenotypical, morphometric analysis. The new feature for the genotype description is the microsatellite analysis.


The applied method offers data for very detailed discrimination of distances among populations.

The results were compared and completed by morphometric analysis of wings using DAWINO method. The method of microsatellite analyzes is a good tool for very detailed discrimination of distances among populations.

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Non-Apis bees

Symposium organized by Dorothea Brückner/Marinus Sommeijer

BEE DIVERSITY AND CROP POLLINATION SERVICES IN FRAGMENTED LANDSCAPES

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Human destruction and fragmentation of natural or seminatural habitats and the creation of intensively managed agricultural habitats are major causes for the loss of biodiversity in terrestrial ecosystems. However, it is not well understood to which extent and at which spatial scales pollinator species respond to the resulting loss of habitats and changes in landscape structure. Further it is unclear how biodiversity change affects ecosystem functioning and services. Crop pollination is an ecosystem service of significant economic value, and there is increasing evidence that wild pollinators contribute to production in several crops. In this context I will present key results and ongoing interdisciplinary research projects.

BUMBLEBEE BEHAVIOURAL AND SENSORY DIVERSITY UNDER THREAT BY INDISCRIMINATE TRADE?

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The European bumblebee species Bombus terrestris has recently become a model to study the evolution of sensory traits and behaviour, because of the intriguing variation that occurs between populations of this species. Island and mainland populations from Corsica, Sardinia, Britain, the Canary Islands, Germany, France, Turkey and Israel differ in spectral tuning of photoreceptors, floral colour preferences, learning behaviour, foraging performance, phenology, and colour coat. As we are beginning to understand the ecological and evolutionary reasons for this diversity, these between-population differences are already under threat because commercial breeders ship colonies from these populations in large numbers to destinations where they are not native. This is because bees of this species are popular with growers of commercial greenhouse crops, such as tomatoes. However, non-native bumblebees might easily establish at non-native localities, interbreeding with native populations. We present a risk assessment of such commercial displacements, and viable strategies to minimise the dangers of population homogenisation.
COMMUNICATION IN EU SOCIAL BEES: COMPARISON OF COLLECTIVE FORAGING

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Social bee foragers can collect food either based on their own decisions, based on information obtained from nest mates or a combination of both. Honeybees, stingless bees, and bumblebees differ strongly in the behavioural mechanisms they have developed to explore for and to exploit food sources and in this talk I would like to try to compare the different groups of social bees using a single coherent concept (see Biesmeijer and de Vries 2001). I will characterize individual foragers as scout, recruit, inspector, reactivated forager, employed forager, unemployed experienced forager, and novice forager. I will emphasise on stingless bees and bumblebees, but compare them to honeybees throughout.

MATING IN BEES: UNIQUE BEHAVIOUR BY WHICH MALES APPLY PHEROMONES ONTO FEMALE ANTENNAE

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In several taxa of bees males have independently evolved modes of mating behaviour through which sex-pheromones are applied directly onto the antennae of females.

In *Anthophora plumipes* the males’ middle legs are elongate and bear long brush-like hairs, but no odour glands. Before mounting the female the male brushes secretions from glands in the abdomen onto his hind legs. They then transfer the odour to the middle legs and finally they brush this secretion during mating onto the female’s antennae.

In megachilid and *Coelioxys* bees of the New and Old World males have modified front legs which bear the odour glands in the tibia and/or in the basitarsus. To apply the contact sex-pheromone, a megachilid male mounts the female, holds the female’s antennae with special structures of his mandibles, covers her compound eyes with blind shields present on his front legs and then secretes the paste like pheromone from pores in his front legs onto the antennae.

In some genera of New and Old World *Xylocopa*, males have similar structures on their front legs and exhibit similar behaviour as described for megachilid bees. However, in some species of *Xylocopa* the middle legs, which are modified to hold the female’s antennae and secrete the odour, are of importance in this mating behaviour.

In several species of the cuckoo bees *Nomada*, males have pores of pheromone glands in various segments of the flagella. During mating the male winds it’s flagella around the female’s antennae and slowly pulls them upwards to apply the paste like secretions onto the female’s antennae. The significance of this chemical communication is discussed.
RECENT SPECIATION WITHIN THE WESTERN EUROPEAN BEES OF THE COLLETES SUCCINCTUS GROUP? A SCENARIO FOR THE EVOLUTION OF C. HALOPHILUS

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The palaearctic Colletes succinctus species group comprises twelve species with three of them occurring in western Europe: C. succinctus (Linnaeus) (oligolege of Calluna and Erica), C. hederae Schmidt & Westrich (oligolege of Hedera) and C. halophilus Verhoeff (oligolege of Asteraceae, especially Aster tripolium). Their close morphological resemblance, the minute genetic differences and distribution patterns suggest that these three species are closely related and probably very recently diverged taxa.

For C. halophilus, an endemic species of the coast of the southern North Sea and the English Channel, a hypothetical scenario of speciation was developed. It is based on the recent postglacial landscape change in the southwestern Netherlands that is assumed to be the centre of origin of this species. The effect of landscape change on the availability of flowers as pollen source and the influence of parasitic cuckoo bees is assumed to be the driving force for the speciation of C. halophilus.

ESTABLISHMENT OF OSMIA LIGNARIA AS AN ORCHARD POLLINATOR IN NORTH AMERICA

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Osmia lignaria is a spring-flying solitary bee that very effectively pollinates fruit tree flowers. Methods have been developed to rear and manage populations of this bee for orchard pollination. The establishment of this or any other agricultural pollinator roughly follows a series of steps. First, information on the basic biology of the species is needed, including its life cycle and developmental biology, nesting behavior, population dynamics and pollinating efficacy. At the same time, the basic biology of the pollinator’s parasites and predators should also be studied, as well as the reproductive biology of the target crop. The information acquired through these studies is combined to design a management system. This system should provide guidelines on rearing methods, nesting materials, population densities required for adequate pollination and control against parasites and predators. The management system should then be tested in field trials, and modified accordingly. Finally, the information should be delivered to the agricultural industry. Only pollinators for which a reliable supply of bees can be secured are likely to become established as crop pollinators. In this presentation, we provide an overview of the process that has led to the establishment of O. lignaria as an orchard pollinator.
Stingless bees are amongst the most important pollinators in tropical and subtropical environments; however, little attention has been given to their potential use as pollinators in confined environments. We have studied the adaptability and pollination efficiency of these species: Trigona nigra, Scaptotrigona pectoralis, Melipona beecheii and Nannotrigona perilampoides.

Here we report results on Nannotrigona perilampoides. We first evaluated its acclimation, foraging behavior, and pollination efficiency using tomato (Lycopersicon esculentum) and habanero pepper (Capsicum chinense) in greenhouses. The pollination efficiency against mechanical vibration (MV) and no pollinator (NP) was compared through the percentage of fruit set, weight of individual fruit, kilograms of fruit produced per square meter, and the number of seed per fruit. In this first study we collected evidence that N. perilampoides performed as well as MV and better than NP.

In a second experiment, we evaluated the efficiency of N. perilampoides on large-scale greenhouse pollination against bumble bees. In Yucatan, the bumble bee Bombus impatiens has been the species mainly used for pollination in greenhouses. In this experiment we also used C. chinense. The results showed that the plants pollinated by N. perilampoides set more fruit and the production in kg per m² was better than those pollinated by B. impatiens mainly as a result of the capacity of stingless bees to continue foraging at high temperature and humidity and because bumble bees caused damage to the flowers.

These results suggest that for neotropical enclosures stingless bees can be a good pollinator alternative compared to temperate bumblebees and hand pollination. More studies need to be conducted to increase the production of colonies for pollination purposes.

The role of ecological infrastructures in pear orchards as food sources functional to pollinators management has been investigated. The researches focused, in particular, on the possible function of early flowering boundaries and edgerows in order to anticipate the release of the pear pollinator Osmia cornuta (Latreille) prior to pear (Pyrus communis Linnaeus cv. ‘Abbé Feté’) blooming. Trials were run in the years 2004-2005 in two orchards, one with a Prunus spinosa Linnaeus edge, the other with strips of Brassica oleracea Linnaeus and Brassica napus Linnaeus, sowed during the previous autumn. Four nesting shelters were placed in each orchard, and 200 females and 400 males were released per shelter. Ten females per shelter, and their nests, were identified. The functionality of the two kinds of early flowering food sources in allowing nesting activity’s initiation was compared in terms of number of larval cells produced in the first part (till 50%) and in the second part (till 100%) of the flowering. The number of cells...
produced in both pear orchards was also compared with data collected in the years 1998-2000, when O. cornuta cocoons had been released at the beginning of pear flowering. Different safe measures were adopted and compared to preserve females from pesticide sprays at the end of pear flowering. Results are discussed.

**BIGGER IS BETTER: INTRASPECIFIC EYE SIZE VARIATION AND LIGHT SENSITIVITY IN BUMBLEBEEs**

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Size variation amongst eusocial bees is most profound in the genus Bombus. It has a strong impact on individual behaviour and colony organization. The goal of this study was to elucidate whether size variation has an impact on morphology and light sensitivity of the visual system of Bombus terrestris. After a comprehensive analysis of the compound eyes and ocella we discovered distinct morphological differences in the eye structure, with larger individuals showing an increase in size and number of ommatidia as well as an increase in ocella diameter. In a behavioural experiment we found that differences in body size have a strong impact upon the light sensitivity of the visual system, with larger bees being able to fly at significantly lower light intensities. These data suggest that larger individuals are able to forage earlier at dawn and later at dusk compared to their smaller nestmates, thus being yet another possible factor contributing to the size related division of labour in bumblebees.

**REARING BOMBUS LAPIDARIUS L. (HYMENOPTERA) IN LABORATORY**

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When a method of laboratory rearing of Bombus terrestris L. was applied as the standard in B. lapidarius, the rearing conditions were fully acceptable for B. lapidarius queens. Ways of starting colonies, their development, numbers of individuals, were similar in both species. The outstanding feature of most lapidarius queens was their willingness to accept the cocoon(s) of B. terrestris and even the very young terrestris worker as a helper in the care of brood at the very beginning of colonies. On the contrary from B. terrestris, lapidarius colonies did not produce young queens after they were made orphan, what in terrestris is the rule.

Lapidarius colonies did produce young queens regularly. Those freshly emerged, fed pollen first to develop their fat body, and from the 6th day onward they were ready to mate. They did mate in comparative small copulation rooms (40 litter aquarium covered with mesh) at the window (light). Males produced in laboratory copulated regularly, whereas those from outside did not.

Unlike in B. terrestris, where currently 70-80 % of queens survived the period of 3-6 months of cold storage, in B. lapidarius 75–87 % of queens died under the same conditions. Moreover those, which survived were hardly able to start colonies. The process of entering the diapauses remains to be solved.
This presentation was possible thanks to the financial support from NAZV CR No: IR440114.

EFFECTS OF DIFFERENT STARTING METHODS ON COLONY FOUNDATION OF BOMBUS TERRESTRIS L. QUEENS

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In extensive commercial rearing, one of the main success criteria is colony foundation ratio of hibernated Bombus terrestris L. queens. The effects of different starting methods on the colony foundation of hibernated B. terrestris queens were investigated. Total of 350 hibernated queens obtained from commercial company were used. Each queen was treated with CO₂ and placed separately in the starting box. Queens were submitted to four starting methods: (qtw) queen with one B. terrestris worker, (qhw) queen with one honey bee worker, (qtp) queen with one B. terrestris pupa and (q) only queen. Queens were fed ad libitum with a sugar syrup and fresh frozen pollen and kept in climate rooms at 27 °C ± 1 and 65 ± 10% R.H. The highest egg laying ratio was found in the qtw group (82.8%) followed by qtp (72.8%), qhw (57.1%) and q (35.7%). In qtw, qtp, qhw and q groups, queens started laying eggs 11.4 ± 0.8, 11.7 ± 1.3, 15.4 ± 0.9 and 20.7 ± 1.7 days respectively after they were placed in the starting boxes. Number of egg cells in the first brood was determined to be an average of 4.4 ± 0.2, 3.5 ± 0.2, 3.3 ± 0.2 and 2.7 ± 0.3 in qtw, qhw, qtp and q groups, respectively. Results showed that adding a B. terrestris worker is the best starting method in bumblebee rearing.

EFFECTS OF WEIGHT OF QUEENS AFTER DIAPAUSE ON COLONY DEVELOPMENT IN THE BUMBLEBEE, BOMBUS TERRESTRIS L. (HYMENOPTERA: APIDAE)

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The effects of weight of queens after diapause on colony development in Bombus terrestris were investigated in this study. The weight of 57 queens was determined to be an average of 0.792±0.012 g after diapause. Approximately 82% of the queens laid eggs and 64% of egg laying queens produced worker. No significant statistical correlation was found between the weight of the queen after diapause and colony development patterns in Bombus terrestris. Only the correlation between the weight of the queen after diapause and the number of workers produced in the first brood was found to be significant (p <0.05, r = 0.42, n = 30). Many factors affect on the variation in colony development patterns and the colony foundation ratio of queens during the year round rearing of Bombus terrestris. However, results showed that the weight of the queens after diapause did not affect colony development in Bombus terrestris.
ABOUT 300bumblebee(Apidae:Bombus)speciesin38subgeneraareknownintheworld.Previousmorphologicalstudiesshowedthat31bumblebeespeciesinneourof16EuropeansubgeneracanbefoundinSlovenia.Thetaimofours studywas to obtaincharacteristicofwingvenation, forassessmentofmorphologicalstructureanddiversity ofbumblebees in Slovenia. Over 350 bumblebees from84differentlocalitiestinSloveniawere collected. Rightsidedforewingswereremoved, scannedandanalysed. The coordinatesof19veincrosseswere measuredandusedinthecalculationof35 characteristics. Speciesmeans andstandarddeviationsofthemorphometriccharacters wereanalysed. Statistical analysis using multivariate discriminant analysis and a cluster analysis ofthe Mahalanobis distancesbetweencentroidsshowedsignificant(P<0,05) differencesbetweenspecies. With discriminant analysis we classified intorightspecies more than96% ofbumblebees. Themostinformativecharacters wasdumb-bellindex (R2 0,70). Wingvenationcharacters couldbealsousefultoolforspeciesdetermination, especiallyforspecieswhicharehardtoidentify.

**ARE SOLITARY BEES AFFECTED WHEN FEEDING ON TRANSGENIC INSECT-RESISTANT CROP PLANTS?**

Roger Konrad and Dirk Babendreier

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Potentialeffects ofgeneticallymodified(GM), insect-resistentcropsonnon-target organisms arewidelydiscussed. Honey bees generallyhave to betestedeffectsofsuchplants ortheirinsecticidalproducts and fewdataarealsoavailableforbumblebees. However, solitary bees have not been tested to date, although theyformaverydiverse groupofpollinators and arebothisecologicalandeconomicallyimportant. Furthermore,theydiffer fromhoneybees invariousaspectsoftheirbiology(e.g.provisioningof developinglarvae).

Wewconductedfeedingassaysunderdefinedindoorconditionswithlarvalsof theredmasonbee Osmia rufa. Larvae were reared either on pollen provisions that were bee-collected on atransgenic insect-resistant oilseed rape (producing the protease inhibitor OC-I) or on bee-collected pollen from control plants where atransgenecompound(i.e. the Bt-toxin Cry1Ab, the lectin GNA and OC-1) had been manually applied in purified form. During larval development and the subsequent hibernation period, life history and viability parameters were recorded. Thedatafromtwoconsecutiveexperimental periods (summer 2005 and 2006) will be combined. Data from 2005 revealedthat 0.1% GNA in the larval diet resulted in a significantly lower food conversion (i.e. conversion of provision weight into larval weight). No significantdeparturefromthecontrolwasobservedforallothertreatmentsandparameters tested. Ourexperimental design proved...
to be suitable for testing direct effects of transgenic plants or their purified insecticidal products on solitary bees and could also be adopted for testing agrochemicals.

**MALE MARKING PHEROMONE OF BOMBUS TERRESTRIS: CHANGES IN COMPOSITION AND ACTIVITY**

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Bombus terrestris is a bumblebee species whose males exhibit patrolling behavior during their mating phase of life. While patrolling, males laid scent marks to attract conspecific females. The scent is produced by the cephalic part of the male labial gland. Little is known about changes in the scent production during the male life span.

Labial glands of males of different age were dissected, extracted with hexane, analyzed and the components were quantified. Chemical analyses revealed significant age-dependent changes. Very young males (0-1 day) posses only traces of compounds in the gland extracts (1-20 µg/gland). During 2-7 consecutive days, amounts of compounds increase rapidly (up to 7 mg/gland) and decrease slowly during the following 30 days (down to 7 µg/gland). Terpenic alcohols (2,3-dihydrofarnesol and geranylcitronellol) appear first in the extract followed by aliphatic compounds (ethyl dodecanoate and hexadecanol). Glands of old males (33 days) contained hydrocarbons and esters of terpenic alcohols such as 2,3-dihydrofarnesyl dodecanoate.

EAG and GC-EAD experiments were performed on antennae of virgin queens. The EAG recording showed maximum responses to extracts of glands of 2-7 days old males. GC-EAD analysis revealed six EAD active components of the labial gland extract. Males’ antennae responded to the same components as those of queens.

Data show that pheromone biosynthesis starts immediately after eclosion and reaches its maximum about 4th posteclosion day. It was also confirmed by the transmission electron microscopy of the cephalic part of the labial gland.

**MULTITEMPORAL INVESTIGATION OF GIANT HONEY BEES (APIS DORSATA) MIGRATION IN CHITWAN AREA (NEPAL) BY MEANS OF REMOTE SENSING**

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The study area is located in the district of Chitwan (Nepal). The Giant Honey Bee swarms (Apis dorsata) migrate in autumn and winter to the southern sub-tropical part of Nepal. The main focus of the inter-disciplinarily project is to analyze the occurrence and distribution of bees (done by field work) together with the spatio-temporal (December, February and April) expansions of the crop fields (by LANDSAT-ETM+), to get out the relationship of bee migration and special food offer. The traditional way of monitoring
by doing field work in a large area with parts far off roads and not easily accessible within the monsoon season is very difficult and expensive, sometimes not even possible. Therefore, a good alternative to get data about these areas is by means of remote sensing.

Due to lack of a public geodata base, the whole range of data acquisition (digital maps, Remote sensing data), pre-processing (building up a GIS data base (digital elevation model, digital maps, land use and land system maps) and analyses within Erdas/Imagine must be applied. The workflow and its results will be documented in the presentation paper.

COMMUNICATION PRECISION OF FORAGERS IN THE STINGLESS BEE SCAPTOTRIGONA MEXICANA

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For eusocial bees the precision of communication of food sources, i.e. the proportion of recruits that reach a communicated food source, is a crucial parameter for colony fitness. However, only little is known about the precision of stingless bee communication systems. Here we examined the impact of experience of workers and distance of the food source on the precision of the food communication system of Scaptotrigona mexicana.

The experiments were conducted by training bees to a three dimensional artificial patch at varying distances from the colony. We recorded the choices of individual recruited foragers, either being newcomers (newly arriving recruits) or reactivated (foragers that had previously visited the feeder). We found that the average precision of newcomers (95.6%±2.61%) was significantly higher (p<0.001) than that of reactivated bees (80.2%±1.12%). This effect, in which the newly arriving bees were significantly more precise, was found at all four experimental distances tested (p=0.004). While this might seem counterintuitive on the first sight, this \"loss\" of precision can be explained by the tendency of experienced workers to explore nearby areas to find new rewarding food sources, after they initially had learned the exact location of the food source. Increasing the distance from the colony had no significant effect on the precision of the foraging bees, neither for newcomers nor for the reactivated ones. Thus our data show that experience, but not the distance of the food source, affected the patch precision of S. mexicana foragers.
HOW BEES GET RID OF ANTS?

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The bees collect the resinous materials from various plants for many purposes. *Apis mellifera* worker bees collect plant resin for plugging cracks and holes in their nest cavity. *Apis florea* and *Apis andreniformis* apply the plant resin around the nesting branches. In contrast to *Apis* species, stingless bees are using wax mixed with abundant propolis to build an envelop (involucrum) surrounding the nest. We designed a bioassay to test the response of the predatory ant species, weaver ants, *Oecophylla smaragdina*, and red wood ants, *Formica polyctena*, toward bee materials from Genus *Apis* and *Trigona*. The bee materials were found to be highly repellent against weaver ants in the raw materials. Interestingly, pentane extracts exhibited repellent activity toward weaver ants whereas the acetone and methanol extracts were no repellent activity. On the contrary, the bee materials resulted in very low repellent activity against red wood ants. Perspectives and directions for further research on the active components are discussed.

DECREASE OF FLIGHT ACTIVITY CAUSED BY VESPA ORIENTALIS AT THE FLIGHT ENTRANCE OF APIS MELLIFERA SYRIACA IN JORDAN

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Vespa orientalis is a threat to beekeeping in the countries of the Near East. *Apis mellifera syriaca*, the indigenous honey bee of Syria, Lebanon, Palestine, Israel and Jordan, is a bee adapted to dry-hot regions and reported to have superior ability to withstand attacks by Vespa orientalis. As a particular reaction, bees are said to commence flight during wasp attacks. We investigated, whether bees would decrease flight activity in response to presenting tethered *V. orientalis* wasps at a distance of 5 to 10 cm to the colony entrance. In two years we investigated a total of 29 *A. m. syriaca* colonies, 16 kept in traditional and 13 kept in modern hives, and of 13 colonies of *A. m. ligustica* in modern hives. Outflying bees were counted over 5 min, then a tethered wasp was slowly waved before the entrance for 5 min, and outflying bees were again counted over the following 5 min. The experiments confirmed previous observations of a reduced flight activity of honey bees elicited by the attack of predatory wasps. The reduction was present in both bee subspecies tested, but it was more pronounced in *A. m. syriaca* (48%) compared to an imported strain of *A. m. ligustica* (22%) indicating a better suitability of the indigenous bees of Jordan, where attacks by *V. orientalis* are a common threat during summer and autumn. The results further indicate, that traditional bee hives (A. m. syriaca, 48%) might be better suited to withstand attacks compared to modern Langstroth hives.
Osmia lignaria is a univoltine, spring-flying solitary bee from North America. Development from egg to adult occurs during spring and summer. Eclosed adults remain in their cocoons through the winter in a diapausing stage, and emerge the next spring as temperatures increase. Chilling is required to complete diapause development. We define pre-wintering as the period during which cocooned adults are still exposed to warm temperatures in late summer and early fall. Because the timing of the onset of cold temperatures in autumn varies widely among years, pre-wintering duration may be highly variable from year to year. Our objective in this study was to establish whether pre-wintering duration affects diapause development and fitness in O. lignaria. We exposed females to three different pre-wintering durations (5, 30, 60 days) to simulate years with early, mid and late winter, and measured respiration rates, ovary maturation, weight loss, fat body depletion and lipid levels throughout pre-wintering and wintering. We also measured survival and vigour at emergence. Pre-wintering duration affected diapause development, and we obtained significant differences among treatments in all variables measured. Females exposed to long pre-wintering periods lost more weight and used up their fat body reserves. These females were less likely to survive the winter or were less vigorous in spring. We discuss the relevance of these results for fitness, nesting activity and the dynamics of O. lignaria populations.
Environmental Hazards to Honey Bees

Symposium organized by Klaus Wallner

INTOXICATION OF HONEYBEES – INTERACTIONS OF PLANT PROTECTION PRODUCTS AND OTHER FACTORS

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In recent years colony losses reiterated in Germany. Besides Varroosis many other causes like other bee diseases, nutrition supply as well as effects of pesticides have been discussed.

A chronic feeding study was conducted to find any indications to what extent negative effects of pesticides in sub-lethal doses can be defined from effects of other stressors (pathogens, drugs, mix of plant protection products, malnutrition of proteins) or if there will be found any interactions or coactions.

In screening programme effects of chronic dietary exposure to sub-lethal doses of the insecticide imidacloprid were studied in honeybees being loaded with a potential stressor (Varroa destructor, Nosema apis, drugs, lack of pollen supply). The results confirm a chronic oral toxicity of imidacloprid at concentrations which have in several previous studies reported to be toxic to bees (100 ppb). However, no indications were found for significant differences in sensitivity to imidacloprid between bees being loaded with different stressors and control bees.

Results confirm previous findings that optimizing of protein supply can soften negative effects of stressors. In addition it became apparent that bees from different colonies of the same apiary which were fed in parallel varied in sensitivity.

Semi-field experiment was conducted to assess the risks of mixing plant protection products by simulating commercial application during blooming on bee colonies foraging in commercial seed dressed rape with potential residues in nectar and pollen.

No adverse effects on mortality or on development of exposed bee colonies had been found when bees foraged on rape of dressed seeds and plants were sprayed with one single plant protection product alone (pyrethroid resp. azol-fungicide) or in combination (tank mix pyrethroid plus azol-fungicide).

From the findings of chronic feeding tests and semi-field test it can be concluded that imidacloprid used as standard seed dressing formulation will pose no risks to honeybees.
PESTICIDE TRANSPORT WITH NECTAR AND POLLEN LOADS FOLLOWING TREATMENTS IN DIFFERENT BLOOMING CULTIVATIONS

J. Pistorius, K. Wallner

see page 146 for abstract

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FRENCH REGULATORY SCHEME AND CONTROLS FOR PESTICIDE USES AND RESIDUES IN APICULTURAL MATRICES

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Twenty five apiaries, evenly distributed in continental France have been followed for 3 years in order to monitor weakness of honey bee colonies (Apis mellifera L.). Five colonies randomly selected in each apiary (total: 125 colonies) were visited four times per year during the whole survey. Depending on the season, the following samples were collected at each visit: pollen from traps, adult bees, foundation wax or honey. This three year study has been completed in 2005. Multiresidue analyses were performed on all of the apicultural matrices in order to detect residues of various pesticides including forbidden substances. Pesticide residues were found in all of apicultural matrices. For example coumaphos residues were found in pollen, carbofuran in honey and fungicides in honey bees.

In pollen, honey and honey bees specific analyses were conducted to search fipronil, imidacloprid and some of their metabolites residues. Fipronil and/or metabolite contents were superior to the limit of detection in 16 samples out of the 81 collected in 2003. Residues of imidacloprid and/or 6-chloronicotinic acid were found in 69% of pollen samples collected in 2003. Imidacloprid contents were quantified in 11 samples out of 81 with values ranging from 1.1 to 5.7 µg/kg.

The different steps of the French regulatory scheme for plant protection including post-release field controls are detailed. The occurrence of the residues found in the present study is commented in regard to these legal requirements.

AN INVESTIGATION OF POTENTIAL LONG-TERM IMPACT OF CLOTHIANIDIN SEED-TREATED CANOLA ON HONEY BEES, APIS MELLIFERA L.

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The past decade has witnessed considerable debate over the impacts of chloronicotinyl insecticides on honey bees, Apis mellifera L. Few studies have investigated potential long-term effects after chloronicotinyl exposure. We conducted a 130-day investigation to ascertain effects on honey bee colonies after exposure to canola grown from seed treated with the chloronicotinyl clothianidin. Colonies were placed in the middle of 1-ha untreated or clothianidin seed-treated canola fields for three weeks during bloom, and thereafter moved to a fall apiary. There were 4 treated and 4 untreated
fields, and 4 colonies per field, giving 32 colonies total. Bee mortality, worker longevity, and brood development were periodically assessed in each colony throughout the study. Samples of honey, beeswax, pollen and nectar were regularly collected from colonies and analyzed for clothianidin residues using HPLC with MS/MS detection. Although sporadic treatment or site effects were found on various dates, essentially no differences in bee mortality, worker longevity, or brood development occurred throughout the study. Weight gains of and honey yields from colonies in clothianidin-treated fields were not significantly different from those in control fields. While clothianidin residues were detected in honey, nectar, and pollen from colonies in clothianidin treated fields, maximum clothianidin concentrations detected were 8- to 22-fold below the NOAEC. The results suggest that honey bee colonies will be unaffected by exposure to clothianidin seed-treated canola during bloom.

SUBLETHAL EFFECTS OF FIPRONIL ON THE ABILITY OF FORAGERS TO ORIENTATE IN A COMPLEX MAZE

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The insecticide fipronil was recently suspected to disorientate honeybees during their returning flight to the hive. In response to whether fipronil may disorientate foragers, its impact on orientation in a maze under outdoor conditions was examined. The maze consisted of a 4 × 5 matrix of identical cubic boxes, with each side carrying a hole in its centre where bees crossed (Zhang et al., 1996). Bees had to fly through a sequence of nine boxes to reach the goal – a feeder containing a reward of sugar solution. During training, the foragers were taught to associate a green mark to the reward that was moved step by step along the path. After foragers were trained and individually marked, their ability to find their way according to the presence or the absence of green marks was measured. Along the path, three boxes constituted a decision point: the bee had to choose between a marked hole (correct path) and unmarked hole leading to dead end. Correct and wrong decisions, turn back, and course duration were recorded. Training and test phases were carried out following two feeding conditions: syrup delivered in a feeder placed outside the maze (before and after treatment); syrup added with 1 µg kg-1 (quantified by HPLC-MS) fipronil (treatment). Data of each period were obtained from different bees.

The rate of foragers entering the maze, and so responding to the mark placed at the entrance, was largely reduced in fipronil-fed animals. Before and after treatment, 86-89% of bees equally flew through the whole path and arrived to the goal without mistakes (P=0.35). The rate of fipronil-treated bees making path without mistakes decreased to 60%. The performance was lower than controls (P<0.01). In parallel, the rate of bees with unsuccessful searches for goal notably increased with treatment (34% in treated bees versus 4% in control bees). So, the orientation capacities of foragers in a complex maze were highly affected by fipronil.
ASSESSING THE TOXICITY OF SYSTEMIC PESTICIDES TO HONEYBEE WORKERS AND LARVAE - ARE ADDITIONAL TESTS NEEDED?

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The systemic properties of some pesticides used in agriculture and horticulture (e.g. granules, seed treatments, soil drenches) means that the laboratory-based acute toxicity studies undertaken for honeybee risk assessment may be less relevant for assessing the potential for adverse effects in the field. Pesticides with systemic properties may be expressed in pollen or nectar and are thus available to foraging bees at low levels but for longer periods of time depending on the flowering of the crop. Foraging bees returning to the hive may also bring contaminated nectar and pollen used to feed brood within the colony. Current OECD test guidelines only allow for short term exposure of adult worker bees (a single contact dose or a 4 hr oral exposure) with assessments of mortality for up to 48 hrs. The relationship between the toxicity of such acute exposures and chronic toxicity in honeybees is not well established. Chronic (10 day) exposure studies have been undertaken for a limited range of chemicals but with widely differing results. This paper will consider the design of a suitable 10-day LC50 test procedure and assessment of the toxicity of a range of chemicals to worker bees. It also will consider data generated toxicity of pesticides to honeybee larvae to determine whether they are protected by current adult honeybee studies.

A NEW LARVAL IN VITRO REARING METHOD TO TEST EFFECTS OF PESTICIDES ON HONEY BEE BROOD

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A new in vitro rearing method of honey bee larvae was devised to assess the effect on brood of any substance that can reach the hive with nectar and pollen. This method could be used in the risk assessment process since it meets with the following characteristics:

- low mortality rate when no treatment is applied,
- standardisation of the test,
- easiness of carrying out,
- sensitiveness to treatment,
- precise control of the ingested doses of diet and pesticide,
- control of larval mortality for each larval instar,
- control of pre pupae weight,
- measurement of adult emergence rate.

The method enables the study of 1/ lethal effects (calculation of LD50) 2/ sublethal effects (prepubal weight, duration of development, adult morphology and behaviour). The method can be used either to study acute effects by applying contaminated diet to one particular instar, or to investigate chronic effects by providing each day the larvae with the test substance.

The use of our rearing method is illustrated with two insecticides:
- Dimethoate considered as a reference insecticide in toxicological tests on adults,
- Diflubenzuron which belongs to the IGR group.
EFFECTS OF FIPRONIL IN HONEYBEES UNDER SEMI-FIELD CONDITIONS

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We have studied the effects of insecticide fipronil in the honeybee (Apis mellifera L.) in semi-field conditions. Each modality was conducted twice, with a colony of about 20 000 workers and a fertile 1-year-old queen, set in a tunnel. The food was delivered with two artificial feeders filled with sucrose solution and multi-floral pollen contaminated (1 ppm, 100 or 10 ppb) or not by fipronil. Any dead bees found on the ground were counted daily. All anomalies in development and behavior of the honeybee colonies were recorded. The colonies were visited to assess brood surface and food quantities (honey and pollen). Larval development was measured using the method of Oomen et al. (1992). No impact of fipronil at 10 ppb was found on the survey, the foraging activity of workers adults and on larval stages, whilst impact was found with others concentrations. Lethal effect on workers bees were observed with two higher concentrations. The contamination of pollen with fipronil at 1 ppm induced a drastic decrease in the foraging activity on the food source. Negative effects of fipronil were also noted on larval stages.

EFFECT OF BT-CORN POLLEN ON THE DEVELOPMENT OF HONEYBEE LARVAE AND RESISTANCE OF ADULT WORKERS

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For the time being, plantation of GM crop has been suspended in Hungary. Supposed coexistancy of transgenic and isogenic plants is a new chaillange for bee breeding and honey production.

According to recent reports, CryAb toxin expressed in pollen by MON 810 corn, does not cause acute toxicity and not reduce development of hypopharingeal gland of workerbees either (Ramirez-Romero et al., 2005). However no data are available about the effect of Bt-pollen to larval development in vitro and resistance of honeybees.

In our experiments, larval food containing 10 % of Bt corn pollen, 10 % of fresh bee bread was offered in royal jelly to 4 day old larvae maintained in U-shape microplates in vitro. The plates were incubated at 35 °C with 90 % relative humidity. The same feed, but with isogenic corn pollen was given to controls. Controls fed with fresh bee bread containing feed was also applied. On the 8th day, before pupation, the larvae were removed, weighed, and deepfreezed for toxin analysis.

Adult worker bees were placed into wire cages by 20 in each and maintained in incubator at 25 °C and 90 % relative humidity. As feed they were given honey twice a day, that contained 10 % Bt-corn pollen and 10 % fresh bee bread. The feed of the controls contained isogenic corn pollen. During 4 week period, mortality rates were registered and Nosema spore analysis of dead bees was performed.

According to our preliminary results, Bt-corn pollen containing feed seems to slightly reduce in vitro larval development. Further more we noticed reduced survival rate and increased Nosema spore count in caged, Bt-pollen fed adult bees.
IMIDACLOPRID AND BEE MORTALITY IN FRANCE

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In 1997, several beekeepers reported that colony death and weakness were the consequence of the use of imidacloprid for seed dressing. This product is indeed very toxic for bees as confirmed by laboratory and semi-field tests. However, several field studies were conducted in France and elsewhere with colonies kept close to cultures issued from seeds dressed with imidacloprid. None has shown any adverse effect of this product. This has been confirmed by some beekeepers that testified that they suffered no adverse effect from such cultures. Additionally, observations in France revealed imidacloprid in pollen loads of several surveyed apiaries with no acute adverse effects on them.

An expert panel gathered by the French Ministry of agriculture studied several scenarios of contamination of bee colonies by imidacloprid. Experts concluded that several PEC/PNEC ratios were very preoccupying. However, the experiment we had conducted on colonies for mimicking the natural consumption of contaminated nectar (the most risky scenario according to the expert panel) did not show any adverse effect on the tested colonies. This apparent discrepancy probably originates from the security margins used in the above mentioned risk analysis.

We conclude that, if contamination by imidacloprid from sunflower cultures issued from treated seeds may have occurred simultaneously with a period of colony losses as described by several French bee-keepers, such occurrence has not been observed systematically, and no negative impact on bee colonies of the use of Gaucho® has been experimentally demonstrated in the field.

THE PRAGMATISM OF HONEY BEES AS ENVIRONMENTAL BIOINDICATORS

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The goal of environmental pollution control is the protection of human health. Most of applied monitoring methodologies are based on chemical-physical procedures that provide, through their high analytic capacity, exact data regarding the concentration of contaminants in the environment. However, this information is not complete because only a part of the pollution has a toxicological importance for the organisms. There is no data about assimilable quantity or biological effects of the pollutants. For this purpose the biological indicators (e.g. honey bees) can be very useful. They can respond, in accordance to the nature of the contaminant, with population variations, behaviour changes or they can bio-accumulate it, by intercepting and assimilating the bio-available fraction. Anyway these assumptions are not considered by the environmental control agencies probably because the evaluation of bioindicator goodness (particularly honey bees), is based only on the comparison with chemical-physical instruments or with environmental markers. This way, for example the fact that honey bees are present and visit all the environmental sectors, is not taken into account. Different methods and
Instruments, indeed, in relation to their prerogatives, find only a part of the environmental pollution that not necessarily must coincide. The chemical-physical system can be defined as eidetic, because it finds the total amount of the contaminants, while the honey bees find the contaminants in pragmatic way, i.e. considering the bioavailability fraction and the related effects. Integrated monitoring is required to obtain more complete information about environmental pollution.

**HONEYBEES - BIOINDICATORS IN A HEAVY METALS POLLUTED AREA**

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Substances hazardous to bee (e.g. heavy metals, pesticides, agrochemicals etc.) induce changes in bee activities or colony performance. Industrial exhaust gases contain a variety of mineral acids and heavy metals. The aim of this study was to investigate the cadmium, lead and zinc levels in sample of honeybees, mellifera flora and honey in a polluted area from Romania compared with the ones in the clean area. The heavy metals pollution was produced by a non-ferrous metal processing plant. 30 samples of honeybees, 25 samples of mellifera flora and 20 samples of honey were analysed. The samples were harvested from hives situated at 10-15 km away from the plant.

The concentration of cadmium, lead and zinc were measured by atomic absorption spectroscopy after mineralization of the samples in a microwave digestion system.

The high amounts of heavy metals were found in honeybees comparing to the control area. The mean level of Cd was 6,2 mg/kg, Pb level 15,5 mg/kg and Zn level 229 mg/kg comparing with 0,01 mg/kg Cd, 0,8 mg/kg Pb and 3,1 mg/kg Zn in the clean area. In mellifera flora samples the metal levels were higher than in honeybees while in the honey samples the concentrations of heavy metals were higher than in unpolluted area but under the allowable limits.

The results indicated that heavy metals originating from immissions generated a persistent contamination which induced a high absorption of pollutants into bee bodies during foraging. Honeybees can be considered representative bioindicators of environmental pollution.

**A CASE OF ACUTE INTOXICATION WITH CARBOFURAN IN BEES**

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In a morning the beekeeper of an individual farm observed a high mortality of his colonies. He found an excessive number of dead bees in the front of all hives. The apiary was situated in the backyard near a rape plantation. Because the damages were 100% from bees colonies he suspected that there were effectuated phytosanitary treatments to the plants. To establish the cause of the death he called the veterinarian who decided to sent immediately bees and rape samples for toxicological investigations.
Due to the placement of the hives and rapid death of the bees chemical analysis were first performed to identify fipronil (an usual pesticide for rape treatments in our country), organophosphorates, organochlorates and piretroids but the results were negative both for bees and plant samples. Then the analysis were done for another toxic compounds and it was chosen the identification of carbofuran. First step to determinate this compound was done using thin layer chromatography. Characteristic spots developed on the thin layer plate indicated the presence of carbofuran only in bees samples. The confirmation of carbofuran involved GC/MS technique which showed typical ions and retention time for this compound in bees and in spiked bees samples with carbofuran.

The death of the bees was an acute intoxication with carbofuran, a highly toxic compound for animals, birds, insects and fish.

Our results were sent to the beekeeper and to the police department to make investigations as to find of the suspect person.

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**THE USE OF CHLORFENVINFOS: A MATTER OF CONCERN FOR ITALIAN BEEKEEPING**

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The use of some products containing chlorfenvinfos, as Birlane® and Supona®, in the control of Varroa destructor infestation of honeybees is a diffuse practice among Italian beekeepers. The use of these products is in contrast with the Community legislation that not includes chlorfenvinfos as active substance in any authorized veterinary medicinal products intended for beekeeping (Regulation (CE) 2377/90). Furthermore, in Italy, any authorization of commercial products containing this active ingredient has been lifted to the end of 2003 (Regulation (CE) 2076/2002).

Actually Birlane® is easily available on illegal market and its use is much widespread, mainly due to its high efficacy against Varroa-mite. As occurs for other not authorized products, e.g. antibiotics, the use of chlorfenvinfos possesses great risks to Italian honey production. In fact, the presence of residues can cause, not only the withdrawal of the product from sale, but also a negative impact on the entire Italian beekeeping sector. Pharmacoresistance of Varroa-mite against organophosphates should also be considered.

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**IS THE BACTERIAL COMMUNITY OF HONEY BEE INTESTINES AFFECTED BY THE CONSUMPTION OF INSECTICIDAL PROTEINS?**

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Honey bees are important non-target species when the risks of insect resistant, transgenic plants are being assessed. Proteins expressed by these plants generally affect the intestinal tract of sensitive insects. We here tested whether the intestinal microflora of honey bees is affected by the consumption of transgenic pollen or insecticidal proteins and whether it is a good indicator for demonstrating sub-lethal effects on this non-target
organism. We used a cultivation-independent approach based on PCR-amplified partial small subunit rRNA genes and genetic profiling by terminal restriction fragment length polymorphism (T-RFLP) to characterize the bacterial community of adult honeybee intestines. Bees were fed with Bt pollen (transgenic maize cultivar MON 810), Bt toxin (Cry1Ab) or the Kunitz Soybean trypsin inhibitor (SBTI) for the first ten days of their life. In addition, we also analyzed free-flying bees from two locations in Switzerland collected at two different times during the season. Neither the Bt pollen treatment nor high concentrations of Cry1Ab affected bacterial community in honeybee intestines significantly. In contrast, bees that were fed with high concentrations of SBTI had a significantly reduced number of bacteria in their midgut. However, in the field collected bees, changes in bacterial community were also found in relation to location or season. Since changes in the bacterial community were only observed at the high concentration of SBTI tested, which bees are unlikely to consume under natural conditions, we expect that these changes would not have detrimental effects on the health of honey bee colonies.

NEW RISK ASSESSMENT OF HONEYBEES’ INTOXICATION TO SYSTEMIC INSECTICIDES: THE CASE OF IMIDACLOPRID

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Systemic insecticides, which are applied as seed formula and thereafter permanently present in the pollen and nectar of plants present a risk to honeybees which collect and consume pollen and nectar during their entire life. However, the actual directives (EC-91/414) cannot assess properly the risk posed by systemic insecticides because it is based on HQ (Hazard Quotient), a value that does not take into account the application mode of these insecticides (i.e. not sprayed and not limited in time) and the biology of a honeybee colony (i.e. a superorganism). In order to overcome this lack, we propose a new risk assessment approach derived from various intoxication scenarios with the example of honeybees and imidacloprid, a systemic insecticide. This approach is based on the new and existing chemical substances directive which compares levels of exposure (PEC-Predicted Environmental Concentration) to levels of toxicity (PNEC-Predicted No Effect Concentration) of chemicals to organisms in order to protect ecosystems. PNECs are determined from the available data found in the literature on acute, chronic, and sublethal toxicities of imidacloprid to honeybees, to which specific assessment factors are applied. PECs are calculated with the known amounts of contaminated pollen and nectar (e.g. 1.9 ppb in sunflower nectar and 3.4 ppb in sunflower and maize pollens) consumed by different categories of honeybees. Results highlight a risk for all categories of honey bees, in particular for hive bees. New perspectives are given to better assess the risk posed by systemic insecticides to honey bees.
TESTED METHODS FOR THE USE OF HONEYBEES AS BIOINDICATORS

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The CNRS team is developing tested methods for the use of honeybees as bioindicators to detect and monitor (i) temporal and spatial changes in plant diversity and (ii) various sources of environmental pollution. This work is conducted within the consortium FRAGILE that includes several institutions (CNRS, Université de Versailles St Quentin-en-Yvelines, Université Paris XIII, Bergerie Nationale) which objective is to design proper indicators, based on a multi-disciplinary approach, to maintain sustainable use of biodiversity in the region of Ile-de-France (IDF), around Paris. To test these methods, behavioural (honeybees activity and dysfunction), palynologic, chemical (pollen dosages), genetics (populations heterozygosis, stress proteins) and morphometrics (fluctuating asymetry, malformations) measurements are conducted at two contrasted sites – an undisturbed area comprising forest and grasslands and - a disturbed agricultural location. Data is analysed with available expert systems, and methods are automated to generalise the use of honeybees as indicators of plant diversity and ecosystem pollution and dysfunction.

CONSEQUENCES OF A HIVE TREATMENT WITH TETRACYCLINE ON THE QUALITY OF THE HONEY

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Tetracyclines (TCs) are used to control bacterial diseases such as European and American foulbrood, which may cause severe losses in honeybee population and honey production. By using 24 hives randomly distributed into four groups of six hives, this study was performed to measure the occurrence of tetracycline hydrochloride (TC) residues in honey following two types of TC application. Two groups of colonies were treated 3 times with 0.5 g of TC in 1 litre of syrup (group S) or in 10 g of powdered sugar (group P). Six hives of a first control group (C) fed with untreated syrup were installed at 20 and 45 metres from groups S and P respectively. A second control group (DC) was set up 3 km away. Honey was sampled at different times from all hives and was analysed to follow the persistence and diffusion of TC residues into the apiary. One day after the last application, the mean TC concentration in brood chamber honey was ten times higher in group S (40.7 mg.kg-1) than in group P (4.34 mg.kg-1). After 146 days, the mean TC concentration in harvested honey was 1.54, 0.35, and 0.15 mg.kg-1 for groups S, P and C respectively. The control group C had been contaminated by drifting from treated hives. In all hives of group DC, no residues were detected at any time of the study. The half-life time of TC in honey from supers was similar in groups C, S and P and was equal to 65 days. These results show that the TC must be used with precaution in honey production.
Environmental Hazards to Honey bees

TRANSFER AND ACCUMULATION OF HEAVY METALS FROM COTAMINATED SOILS INTO THE NESTS OF BOMBUS TERRESTRIS L. (HYMENOPTERA, APOIDEA) AND LITTLE HIVES OF APIS MELLIFERA L. (HYMENOPTERA, APOIDEA)

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We show the results of a study, in which we tried to find out, if the different components of the nests of Bombus terrestris and the hives of Apis mellifera can be used as a bioindikator for the heavy metal contamination of soils.

DEATH RATE AND WEAKENING OF HONEYBEES IN THE SOUTH PART OF BELGIUM

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For several years, abnormal high death rate and weakening of honeybee (Apis mellifera L.) have been very often observed in the south part of Belgium (Walloon Region). Although two new insecticides, imidacloprid and fipronil, are regarded as being the single cause of the honeybee collapse, many multifactorial studies carried out in Europe and in North America clearly highlight many factors of risk: parasites, diseases, climate, food resources, low farmland biodiversity. The honeybees collapse stigmatizes a deep uneasiness at apiarian world confronted with a more and more anthropized environment.

Then, an exploratory multifactorial study was set up in order to discover the factors of risks which are likely to influence the vitality of the bees in the south part of Belgium. In this project, we study pesticides, pathologies and environment (nutritive resources, climates, etc.).

A list of pesticides including both product types used in apiculture and for the surrounding agriculture has been set up and quantification of residues of those pesticides (about 50 analytes) in honey, beeswax and bees were carried out.

The pathologies were studied combining field observations, microscopic, classic microbiological and molecular approaches.

Finally, the correlation between the honeybee colonies mortality and the presence of pesticides, pathologies and the environment were analyzed.

SUB-LETHAL EFFECTS OF PESTICIDES

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Pesticides may affect honeybees by causing direct mortality or sub-lethal effects which may affect the individual and thus the colony. Effects at the level of the individual may include, for instance, decreased ability to return to the colony and may result in effects at the colony level such as reductions in colony strength. In the last few years,
evaluation and assessment of possible sublethal effects of pesticides on bees has been increasingly discussed by scientists and regulatory authorities. This discussion has been enhanced by allegations, particularly in France, that exposure of honeybees to systemic insecticides may result in sublethal effects directly related to the reported colony losses. Novel study designs and data requirements have subsequently been proposed to test for sublethal effects on individual honeybees, in particular cases or even on a regular basis. This paper will consider whether and, if so, how sublethal effects should be incorporated into risk assessment by considering: What do we mean by a sublethal effect? How do we link sublethal effects to effects at colony level? What should trigger assessment of sublethal effects? How do we include sublethal effects in risk assessments?

CONCENTRATION OF LEAD AND CADMIUM IN BEES AND BEE BREAD

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Studies were carried out in randomly selected apiaries of Warmia and Mazury province. At the turn of June and July the house bees and bee bread were collected from 5 colonies of each apiary in 22 communes of the province. Quantitative determination of lead (Pb) and cadmium (Cd) was done by the method of absorption atomic spectrophotometry.

The presence of lead and cadmium was found in all samples of bees and bee bread. Lead (Pb) content in bees fluctuated between 0.234 mg/kg and 1.165 mg/kg and in bee bread between 0.074 mg/kg and 0.135 mg/kg however cadmium (Cd) was respectively 0.067-0.324 mg/kg and 0.030-0.072 mg/kg. The obtained results indicate the low concentration of lead and cadmium in bee bread. The average content of Pb and Cd in bees from individual communes was about 1.7-12.7 and 1-10.8 times higher than in bee bread. The highest concentrations of Pb (0.932-1.421 mg/kg) and cadmium (0.210-0.324 mg/kg) were ascertained in bees from apiaries situated in central and western part of Warmia and Mazury province. In these communes, agricultural and food industry is concentrated, main transit roads run to the Russian border as well as manufacturing agriculture is developed.
ATTRACTIONNESS OF AVOCADO NECTAR TO HONEY BEES IS REDUCED DUE TO HIGH MINERALS CONTENT

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Honey bees are important avocado pollinators, but even when bee hives are placed in an orchard, bees tend to prefer competing blooms, and avocado pollination is often inadequate. The possible effect of nectar composition on the preferences of bees was tested in this work using three measures: choice between feeders, and dance and learning performance. Since honey is of nectar origin, we used honey in the experiments and we identified avocado honey due to its peresitol concentration, a unique avocado carbohydrate. When choosing between solutions of equal sugar concentration, bees preferred non-avocado honey and sucrose solution to avocado honey. Bees significantly increased their dance rate when non-avocado honey reward was replaced by avocado honey. Bees that were first rewarded with non-avocado honey, avoided the avocado honey that came later. In conditioning of the proboscis extension response, the percentage of bees that learned to associate between odor and reward was lower when the reward was avocado honey than non-avocado honey or sucrose solution. Moreover, an increasing percentage of bees refused to consume the avocado honey during the course of the experiment. Avocado nectar and honey were found to be rich in a wide range of minerals including: potassium, phosphorus, magnesium and sulfur. In a choice experiment with feeders, dissolving potassium and phosphorus in non-avocado honey resulted in reduced attractiveness to bees. These findings highlight the importance of nectar micro-elements, such as minerals, in nectar evaluation by bees, and consequently on learning, choice, and recruitment, which directly affect pollination performance.
ALMOND POLLINATION AND THE USE OF HIVE ENTRANCE POLLEN TRANSFER DEVICES

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During 2005, a shortfall in natural pollination services provided by bees on almonds was quantified in N. Greece. Therefore, in 2006, hive entrance pollen transfer devices were used in order to determine: a) their efficiency in improving cross-pollination on almonds and b) their practical use. A type of soft material was fitted on a hive entrance device on experimental colonies. Four groups of experimental and control colonies were transferred in the crop and every group stayed for 2 days. The efficiency of the hive entrance devices was measured by means of bagging branches with flowers, which were self- or cross-pollinated by hand and fruit set was compared with fruit set on other flowers that had been bagged without pollination and others that were bagged but bee visits were allowed on them every second day. Fruit set on those branches visited by bees from experimental colonies (fitted with hive entrance devices) was higher but not significantly so compared with fruit set on branches visited by bees from control colonies. The design of the hive entrance device used in this experiment found not to be practical as it was not tolerated well by the bees. A different design, tunnel like, was found to be better tolerated but it has not been tested for efficiency yet.

POLLINATORS AS CARRIERS OF BIOCONTROL AGENTS

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Pronubial insects can act as very efficient carriers of beneficial microorganisms for the control of those pathogens whose preferential way of penetration is represented by the flower organs, like Erwinia amylovora and Botrytis cinerea, as examples. Their use as vehicles for BCA can result in a very efficient system also in the case of secondary dissemination between BCA-contaminated and uncontaminated flowers. In the complex flower-pathogen-antagonist-carrier relation the reliability and efficacy of the carrier insect as a pollinator of the target plant is a crucial point. Once the pollinator species has been selected, a BCA’s dispenser system fitted with the pollinator’s needs and behaviour has to be developed. Researches have been run to develop a BCA dispenser system suitable for the habits and the nesting behaviour of the solitary bees Osmia cornuta (Latreille) and O. rufa L. for the prevention and control of the bacterial fire blight on pear and apple, being them good pollinators for these crops. A comparison was run with Apis mellifera L. for which a new dispenser model previously developed was optimized. The functionality of the dispensers was assessed through behavioural observations and evaluation of the amount of active bacterial cells acquired passing by the dispenser and deposited on the flowers.
SEASONAL CHANGES IN THE POLLINATORS’ PERFORMANCES IN TWO POPULATIONS OF MICROMERIEA FRUTICOSA L. (LAMIACEAE) ON MT. CARMEL ISRAEL

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ASSESSING THE DEPENDENCE ON INSECT-MEDIATED POLLINATION OF EUROPEAN AGRICULTURE

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Following the 1998 Sao Paolo declaration on pollinators, the 2001 Decision IV/6 COP5 of the convention of the parties that launched the International Pollinator Initiative stressed the urgent need to assess the economic value of pollinators. Central to such economic assessment is the measure of the level of dependence of crop agronomic and economic yields on insect-mediated pollination. Within the EU-project ALARM (http://www.alarmproject.net), we used a uniform plantwide-based protocol on 5 important entomophilous crops to measure the incidence of insect-mediated pollination and the impact of pollinator decline in abundance and diversity on crop yield and quality. These crops were buckwheat in Poland, cantaloupe in France, field beans in the United Kingdom, spring rape in Sweden and strawberries in Germany. To get a range of pollinator diversity and abundance, for each crop we used 8 to 10 fields located in a gradient of landscape context from mainly intensive agricultural surroundings to mainly natural habitats in order to get a range of pollinating species and population density. Data on pollinator abundance and diversity were recorded as well as yield and crop quality from plants with all their flowers either exposed to passive self-pollination (PSP), or to PSP and 75% of wind-borne pollen, or open-pollinated with free access to insects. The results of this Europe-wide study will be presented and compared with previous such assessments, and conclusions will be drawn in reference to the economic assessment of the value of insect pollinators for European agriculture.

THE POLLINATING INSECTS EFFECT ON SEED YIELD OF SUNFLOWER (HELIANTHUS ANNUUS L.) PLANTATION

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The beekeeping value of sunflower plantation was conducted in Pulawy in the year 2005, together with the response of this species on the lack of pollinating insects. The fruit and seed setting from plants freely accessible for pollinating insects were compared with plants isolated from the bees during the blooming time. The sunflower's blossoms were willingly foraged by the honey bee's workers and
bumble-bees collecting the nectar as well as the pollen and the simultaneously making pollination of blossoms. In the initial and the final phase of blooming we could meet only 1 insect per 1 m², but when the weather conditions were favourable to fly, during middle part of blooming, the density of insects carried out from 3 to 5 per each 1 m². The studied cultivar after isolation of flowers during the blooming developed the fruit with seeds at average from 46.5% of produced flowers. Over 53% of achenes had no seeds. However in case of free access of insects to blossoms, the setting fruits with seeds were on level 85.5% and only less than 15% of achenes were empty. The achenes harvested from both variants of experience were alike shapely. The mass of thousand seeds carried out average 64.8 g and 66.8 g from free pollination and isolation respectively.

The calculated yield of seeds from analyzed experimental surfaces for freely accessible plants for insects carried out 5.72 t per 1 hectare, however for plants isolated through the blooming only 3.31 t per 1 hectare. The real yield of sunflower's seeds gathered by the farmer according combines harvest method carried out 3.05 t per hectare. During the EurBee2006 the two-year results of investigations will be presented.

**MOTIVE OF INSECT POLLINATORS AND BEEKEEPING ON POSTAGE STAMPS**

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More than 300 countries and other territories issued stamps displaying insects. Alongside with butterflies and beetles, bees belong to most popular insect motives portrayed on more than 300 stamps. Bee stamps are often part of series representing native fauna and nature conservation. Bees are also grateful postage motive representing diligence, labor, thrift or technical cooperation, design of bee comb symbolize unity, representing individuality as a single cell. First beekeeping stamps were issued in 19th century, but most of collection items appeared just recently. Besides western honeybee also other representatives of superfamily Apoidea - especially bumblebees and solitary bees – can be found on stamps. Most interesting stamps are inscribed to beekeeping practice, history of beekeeping and traditional hives, bee predators, famous beekeepers or as a memorial of beekeeping congresses and anniversaries.

**IDENTIFICATION OF POLLEN FROM WILD, CULTIVATED AND ORNAMENTAL PLANTS IN LA LAGUNA REGION, MEXICO**

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In La Laguna region (Coahuila and Durango states) in northern Mexico, the anthers of wild, cultivated and ornamental plant species were collected in order to isolate the pollen using the acetolisis technique. The collection is serving as a reference database for identifying plants visited by honeybees and the plants blooming period in
the area. Beginning in the spring of 2002 and through the years 2003, 2004, 2005 and 2006 were obtained, processed and photographed pollen of plants. Plants were photographed, collected and dessicated to preserve in the herbarium. The pollen samples were processed and observed in a microscope Olympus modelo BH-2, connected to a TV screen. Each pollen grain was measured with objective micrometer immersion oil at 100X. Pollen grains were photographed at 400X and 1000X with a reflex Minolta SRT 101 camera, Rokkor lens PF 58 mm mounted in a tripod using color slide film ASA 100, f/9 obturation at a ½ second speed. At least 2 images were obtained at different angle and scanned in a 3500C HP scanner. The collection has, up to date, 209 – wild, cultivated and ornamentals- species. We found the families Acanthaceae, Agavaceae, Aloaceae, Anacardiaceae, Ampelidaceae, Apocinaceae, Asteraceae, Bignonaceae, Bombacaeae, Boraginaceae, Brassicaceae, Cactaceae, Canabinaceae, Caesalpiniaceae, Chenopodiaceae, Convolvulaceae, Casuarinaceae, Cucurbitaceae, Drupaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Juglandaceae, Labiatae, Lamiaceae, Laureaceae, Liliaceae, Malvaceae, Meliaceae, Mimosaceae, Moraceae, Musaceae, Myrtaceae, Nyctaginaceae, Oleaceae, Palmaceae, Papaveraceae, Pittosporaceae, Plumbaginaceae, Poaceae, Polygonaceae, Portulacaceae, Punicaceae, Quenopodiaceae, Ranunculaceae, Rosaceae, Rutaceae, Salicaceae, Solanaceae, Tamaricaceae, Umbelliferae, Verbenaceae and Zygophyllaceae.

ANALYSIS OF TRANSGENIC POLLEN IN RAPE HONEY WITH REAL TIME PCR


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Today North America, especially Canada, cultivate a high amount of genetically modified rapeseed (75 % of the acreage), whereas in Europe transgenic rapeseed is restricted to field trials. Even in honey, pollen of transgenic rapeseed can be detected by sensitive nucleic acid analysis. So far, transgenic rapeseed has only been detected in Canadian honey¹. However, unavoidable and unintentional contamination with transgenic rapeseed pollen can also happen in honey produced within the EU. In the past years detection of transgenic rapeseed used for cultivation within southern Germany has been reported, although only traces could be found². The Standing Committee on the Food Chain and Animal Health considered honey as an animal product not coming under the scope of the regulation [1829/2003/EC] and stated that honey does not need to be labelled “provided that the proportion of GM pollen in the honey is no higher than 0,9 per cent”.

In this publication, the analytical “state of the art”, including possibilities and limits of the analysis of rape honey by real-time PCR is described. An optimised method for the extraction of DNA from pollen in honey is shown. Different real-time PCR systems for the quantification of DNA specific for rapeseed as a reference gene were evaluated. The amount of amplifiable DNA from rapeseed determines the detection resp. quantification limits (in terms of percent values, proportion of gmo DNA vs. rapeseed DNA) for the transgenic DNA-sequences⁴. The data shown demonstrate the detectability of gm sequences down to gmo proportions in the range of 0,1 to 0,9 per cent. This is possible, provided that there is more than 50 per cent rape pollen in proportion to the
whole pollen amount. A correlation between rape pollen ratio and the detection limit (relative, per cent gmo-DNA) was evident. Two construct-specific sequences present in glyphosate- (e.g. GT 73) resp. glufosinolate tolerant (e.g. liberty link) rapeseed lines were used for the relative quantification of transgenic DNA vs. rapeseed-DNA in honey5.

In the future, sensitive methods for the analysis of transgenic rape in honey will not only be significant for food analysis. It can also be used as a tool for monitoring programs of field releases or coexistence studies of transgenic and conventional plants.

SPATIAL AND TEMPORAL DISTRIBUTION OF HONEYBEE FORAGERS IN A CANTALOUPE FIELD WITH DIFFERENT COLONY DENSITIES

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The field trial was carried out in La Laguna production region (near Torreón, Coahuila) in northern Mexico, during summer 2003. The objective of this work was to determine the temporal and spatial honeybee (Apis mellifera L.) distribution in cantaloupe (Cucumis melo L.) fields with different colony densities. Starting in the second blooming week, honeybee colony densities were increased by adding one to five colonies per hectare one day before every observation day. Honeybee colonies were uniformly distributed adjacent to a cantaloupe field. In five randomly selected rows, transects 10 m long were marked at 25, 50, 75 and 100 m distances from the center of the apiary. Foraging bees were counted every 30 minutes from 7:30 to 19:30 hr. Significant differences (P < 0.001) in the number of pollinating honeybees were found among distances evaluated, with the highest density at 50 m (7.2a), intermediate at 25 m (6.6ab) and 75 m (6.7ab), and the lowest at 100 m (5.3c). Foraging honeybees numbers varied temporally (P < 0.0001), observing the highest density from 10:00 hr to 15:00 hr, with a peak at 11:00 hr. Number of colonies affected the honeybee density (P < 0.0001). The maximum bee number per transect (6.08a) was observed with three colonies per hectare; therefore, this number of bee colonies could be considered the optimum number for cantaloupe pollination.

CHANGES OF THE POLLEN SPECTRUM OF HONEYS DURING ITS RIPENING IN THE BEEHIVE

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The obtainment of unifloral honeys in Polish environmental conditions is difficult due to not very abundant and interrelated nectar flows. Foraging bees bring to unripe honey the nectar from other nectariferous plants blooming later what reduces the proportional contribution of dominant pollen of the plant giving unifloral honey. Research carried on in our Institute confirmed that percentage of the dominant pollen in
unripe honeys (samples received during the nectar flow) is higher than in ripe honeys (samples of capped honey). The samples of honey from the same beehive were taken. Leaving the honey to its natural ripening in combs caused decrease the content of the dominant pollen even for over 20%, what in extreme cases can lead to decrease the percentage of dominant pollen below minimum required for unifloral honeys. Therefore it seems advisable to harvest unifloral honeys before the end of the nectar flow in spite of high water content in its. Other research carried on in the Institute in Pulawy proved that quality parameters of the honey do not decrease in the dehydratation process.

RELATIONS BETWEEN APIS MELLIFERA L. AND TULIP TREE (LIRIODENDRON TULIPIFERA L.)
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Liriodendron tulipifera L., native to North-Eastern America where is present in hardwood forests, in the XVIII century has been introduced in Italy as ornamental plant. It is cultivated for its wood, which has a high commercial value and a good versatility. This plant is an important source of nectar for honeybees in North America. In relation to its spreading in Italy and to the lack of knowledge about its apicultural interest in the European region, we examined the relations between Apis mellifera L. and tulip tree.

The research has been carried out in 2004 – 2005 in seven stations of Piedmont (Italy), localized in Turin, near Turin and in Padova (Venetia). Foraging behaviour of the honeybees, towards this plant and accompanying blossomings, has been examined; the nectar sugar concentration has been analysed through refractometer.

From the investigations on the foraging activity of nectar and pollen, the bees frequency on tulip tree was not steady. During L. tulipifera blooming period in May, Robinia pseudoacacia L., very common in North Italy, was much more visited than it. The bees frequency on tulip tree flowers depended on flowers age and weather conditions. Statistical analyses were done to find relations among nectar production and sugar concentration with respect to climatic factors as relative humidity, rainfall and temperature.

At start of flowering the flowers have been produced abundant nectar at low sugar concentration, around 19%, while on second day flowers usually have been produced less nectar, but the sugar percentage reached even more than 60%. The flower wide shape allowed other botanical species pollens to get inside L. tulipifera nectar. This research confirms the importance of tulip tree for bees, especially in urban areas.
THE EFFECT OF POLLEN TRAPS ON THE POLLEN PREFERENCES OF HONEYBEES

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Pollen traps are commonly used to record the bee flora of an area. However, little is known about their effect on the pollen preferences of bees.

In order to study the influence of pollen traps in the foraging behaviour of bees, we evaluated the contribution of pollen sources in colonies of Apis mellifera L. First we fitted pollen traps to the entrance of ten hives and we measured the contribution of the trapped pollen sources during a five-day period. Then, we removed all beebread from half of the colonies (treated group) in order to raise the pollen needs and create conditions similar to those that pollen traps cause to colonies. Five days later we collected again the trapped pollen from the treated and the control group and we estimated the contribution of the pollen sources.

Even though the amount of trapped pollen rose significantly in the treated colonies, there were no statistically significant differences relative to the number or the contribution of the pollen sources between the two groups.

We concluded that the use of pollen traps to record the pollen flora of an area does not influence the pollen preferences of the bees and the accuracy of the results.

THE EFFECTS OF HONEYBEE POLLINATION ON CANOLA WITH AND WITHOUT BOTTOM POLLEN TRAP HIVES IN CAGES

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Canola is not only consumed as vegetable oil but also alternative energy as biodiesel in recent years. Canola is also an important bee plant for spring build up. The effects of honeybee foragers in two different hives were tested in order to determine seed formation and production qualities on canola.

Honeybee (Apis mellifera anatoliaca) and canola (Brassica napus) Pulsar winter variety were used in the experiments. Small honeybee colonies were placed in mini hives containing approximately 2000 bees in regular and bottom pollen trap hives. Experimental 4 groups each containing 4 cages that are 5m long, 4m wide, 2.5m height with insect screen were used. Each experimental unit contains four fields: 1) an open field, 2) a cage without honeybee pollinators, 3) a cage with honeybee foragers in a regular mini hive and 4) a cage with honeybee foragers in a bottom pollen trap hive. Fields 1 and 2 represent experimental controls.

1000 seed weight, plant weight, branch no., pod no., seed production per plant, and harvest index were determined. Protein and oil content per seed were determined by Kjeldahl and Soxhlet apparatus. Seed production per 1000 m² (no.1:320.5 kg, no.2:182.3 kg, no.3:236.0 kg and no.4:235.7 kg for two year averages) pod no., and seed production per plant were found significant and highest values were obtained from open field and second with bees in regular hives. 1000 seed weight, plant weight, branch no. per plant, harvest index, seed protein and oil quantity per seed were found insignificant.
Honeybee pollination increase seed production significantly but not protein and oil range per seed.

**TAGETES L. AS A POLLEN SOURCE FOR HONEYBEES**

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During the seasons 1994, 1996, 2004-2005 the investigations were carried out on two species of Tagetes L. There were T. erecta L. and T. patula L. The purpose of the study was to examine their blooming and pollen productivity. The flowering observations were conducted when the first flower heads started to bloom and lasted till the last days of flowering. The number of inflorescences per one plant as well as the number of flowers per flower head were determined. Pollen productivity was estimated by the ether Warakomska’s method (1972) with modification.

The length of flowering period of Tagetes was on average from 2.5 to 3.5 months. In all years of the study T. patula started its flowering a few weeks earlier (in mid June) than T. erecta. Blooming of both taxa endured till the first frosts. During vegetation season one plant of T. erecta developed on average 48.7 flower heads, whereas T. patula 88.5. There were two types of florets in the inflorescences of both species – ligulate female and tubular bisexual florets, whose contribution reached 47.6% and 52.4% respectively for T. erecta, 43.9% and 56.1% for T. patula.

Pollen grains of Tagetes are round shaped with spiked exine, they are produced only in tubular florets. The average pollen efficiency of 10 florets was similar for both species and hesitated from 2.15 to 3.02 mg for T. erecta and from 1.95 to 3.08 mg for T. patula respectively. The estimated pollen yield per one plant riched the higher value for T. erecta – 1.53–1.65 g, whereas 0.89–1.06 g for T. patula. Flower heads of both species attracted numerous insects, mainly honeybees. The most intensive visitation was recorded at noon hours.

**PHENOTYPE VARIABILITY OF POLLEN GRAINS OF CHESTNUT (CASTANEA SATIVA MILL.)**

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The aim of work was characterization and evaluation of pollen grain morphology on selected genotypes of chestnut (Castanea sativa Mill.). The 23 tested genotypes were from two localities of Slovak Republic, namely from Radošíná and arboretum Mlyňany. Using the scanning electron microscope Tesla BS 301 the morphological analysis was
realized. The detail records of pollen grains, population of developed and undeveloped pollen grains were made at different magnification. The image database is prepared from obtained records. The quantitative parameters are evaluated by using the light microscope Zeiss Axiostar plus. The possible intraspecific differences were examined by the study of pollen grains morphology between selected genotypes of chestnut. This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-026704.

**INTRASPECIFIC VARIABILITY OF POLLEN GRAINS OF COMMON POPPY (PAPAVER SOMNIFERUM L.)**

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In our work was tested a morphological variability of pollen grains on 23 genotypes (old varieties and land races) of common poppy (Papaver somniferum L.). The genotypes were from different areas of Liptov and Spiš in Slovak Republic. Obtained pollen grains from the individual genotype were evaluated by a light (LM) and scanning electron microscopes (SEM). Using the LM Zeiss Axiostar Plus were pollen grains measured in the length of polar axis (µm), the length of equatorial axis (µm), and the shape index by which the pollen grains were classified to shape classes. The thirty pollen grains were evaluated for each selected genotype. The coefficient of variability was 8.5% for the length of polar axis and 7.4% for the length of equatorial axis. Using the SEM Tesla BS 301 the morphology of pollen grains was evaluated. For each genotype was made 8 detailed records of an equatorial and a polar view, a detail of exine surface, and population of developed and undeveloped pollen grains, at the different magnification. The exine surface of poppy pollen grain was characterized as microechinate-perforate, and 3-colporate apertures. In the framework of obtained experiences from pollen grains evaluation by SEM we studied the possibility of common poppy pollen pellets identification. This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-026704.

**THE PRELIMINARY STUDY OF THE INFLUENCE OF POLLINATORS ON FRUCTIFICATION OF TWO CULTIVARS OF LONICERA KAMTSCHATICA (SEVAST.) POJARK.**

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The Lonicera kamtschatica produce edible, early ripening fruits. The characteristic feature is double flowers inflorescences. The inferior ovaries of both flowers are aggregated by sepalous leaves which form homogenous, fleshy coating of multiple fruit. The ripen multiple fruit consists of two false-berries and looks like a single berry.
The investigations on influence of pollinating insects on fructification of Lonicera kamtschatica were undertaken in 2004 in Lublin, Poland. Two cultivars ‘Atut’ and ‘Duet’ were examined. Eight bushes for each cultivar were chosen and two pollination treatments were applied: self-pollination and free-pollination. On every randomly chosen branches flowers and ripen multiple fruits were counted. The mass of ripen multiple fruits from each combination was estimated.

The both examined cultivars show similar reaction to the pollination method applied. The highest percentage of berries was present after free-pollination, 96% on average. The percentage of berries was significantly lower after self-pollination and varied from 16.38% ‘Atut’ to 23.76% ‘Duet’. The highest quality of multiple fruits was present after free-pollination. The mass of multiple fruits set after self pollination under isolators was 30-40% lower then set after pollination by insects. Flowers of investigated plants were mainly foraged by honeybees collecting nectar and pollen and by bumblebees and rarely by solitary bees.

THE POLLEN ABUNDANCE OF SOME EARLY SPRING FLORA OF ANTHROPGENIC HABITATS AS A PART OF APOIDEA FEEDING BAND

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In recent years the lack of food, particularly pollen, connected with destruction of weeds, flora on barrows, waste lands, burnings of waysides become an important risk for pollinators. Honey bees and wild Apoidea collect nectar and pollen from different flowering plants. The pollen abundance, pollinators composition and forage pattern were observed in Lublin, Poland (51°15′N, 22°03′N). In the period between end of March till the mid of May 20 flowering species were identified in ruderal vegetation. Apis mellifera foraged mainly on Tussilago farfara, Taraxacum officinale, Ficaria verna, Barbarea vulgaris and Prunus spinosa (over 80% of pollinators) and bumblebees queens on Lamium purpureum, L. album, while solitary bees foraged on Veronica chamaedrys, Viola odorata, V. canina, Bellis perennis, Cardaria draba, Potentilla arenaria, P. anserina, Euphorbia ciperissias and Glechoma chederacea as well. Tiny flowers of Erophila verna were attracted for Diptera. The highest density of forage taxons were in Senecioni-Tussilaginetum, Cardario drabae-Agropyretum repentis and in communities with Potentilla arenaria and with Euphorbia cyparissias. The forage species occur mainly on the city borders, along the roadsides, on railways slopes.

The average pollen abundance per 100 flowers was 2.25 mg (Glechoma hederacea) 6.8 mg (Cardaria draba), 20.56 mg (Barbarea vulgaris), 65.08 mg (Potentilla anserina)

Early spring flora is very important as food for the adult bees, for brood rearing and especially for females of bumblebees making their new colonies after winter and for solitary bees.
FORAGE PATTERN ON SOLIDAGO L.

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Different wild species are recommended for flow improvement (Szklanowska 1995, Jabłoński 2000, Denisow 2004).

During the course of three years study (2001-2003) on nectar and pollen efficiency of Solidago sp. the detailed observations of some aspects of blooming and insects visits were made. The Solidago gigantea Aiton, S. canadensis L., S. virgaurea L. s.str. i S. hybrida hort. were cultivated on experimental plots in Pulawy (south-eastern Poland). Additionally two species S. gigantea and S. virgaurea were studied in anthropogenic phytocoenoses in Wlostowice near Pulawy.

The blooming of investigated species lasted from the end of July till the end of September in the period of summer flow gap. The pattern of diurnal dynamic of blooming was similar for all studied species. The blossom of disc florets was most intensive between 9.00 and 13.00 (EET). The flowers were visited mainly by nectar and pollen collectors of Apis mellifera, which consisted 90% of pollinators. The participation of different Bombus sp. and solitary bees was similar – 0.6%, Diptera 5.3%, Vespula sp. 3.2%, Lepidoptera and Coleoptera consisted only 0.3% of pollinators. During dry, sunny weather insects foraged on Solidago sp. from 8.00 till 16.00 (17.00) and most intensively in mid-day hours. In pick blooming period (full blooming phase) 30-40 honey bees per 1m² were observed. The pollen loads were brick orange in color and weighted 6-9 mg per pair, on average.

The time of blooming, abundance of nectar and pollen delivered, the intensity of Apis mellifera and wild bees forages, create large possibilities to propagate Solidago sp. on different barrens to improve forage base.

NUTRITIONAL VALUE OF AN IMPORTANT SOUTH AFRICAN BEE POLLEN: THE FRESH, BEE-COLLECTED AND STORED POLLEN OF ALOE GREATHEADII VAR DAVYANA (ASPHODELACEAE)

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The most important indigenous bee plant in South Africa is the winter flowering Aloe greatheadii var daviana, eagerly utilised by migratory beekeepers when other food sources is unavailable. The strong pollen and nectar flow of this aloe is used not only for honey production but also to build up colonies. Fresh, bee-collected and stored pollen was collected and analysed for its nutritional content. Analyses included amino acid and fatty acid composition. Protein content of this pollen is the highest reported yet for South African pollens and the overall nutritional content appears to be superior to that of many other types of pollen. The three types of pollen differed significantly from each other. After collection and storage by honeybees, increases in water content (13 to 21% wet mass) and carbohydrate content (35 to 61% dry mass) were observed, resulting in decreases in crude protein (51 to 28% dry mass) and lipid content (10 to 8% dry mass). Proportions of essential and non-essential amino acids did not differ significantly between the three types of pollen. With the exception of tryptophan, all the essential
amino acids for honeybees were present in equal or higher amounts than the required minimum levels for bee development. Fatty acids comprised a higher proportion of total lipid in fresh than in bee-collected and stored pollen. Concentrations of individual fatty acids in A. greatheadii var daviana pollen were close to values reported for other pollens, the major difference being the high gadoleic acid content. To our knowledge this study is the first to compare the nutritional content of fresh, bee-collected and stored pollen from a single species.

**INFLUENCE OF DIFFERENT POLLEN CONSERVATION CONDITIONS. PRELIMINARY STUDIES**

Pérez Martín, R.A., González Lorente, M., Vela Hortigüela L. and de Lorenzo Carretero, C.


Bee pollen is one of the foodstuffs of the honeybee (Apis mellifera), which collects pollen grains from different flower species. This product is mainly composed by proteins, free aminoacids, lipids, carbohydrates, vitamins, minerals and trace elements. Open air-drying, artificial heating or freezing are the most commonly used methods for conservation of bee pollen, although this product is usually marketed in a dried form. Data about the effects of preservative methods on some pollen properties are scarce in the available literature.

In the present work, preliminary assays have been carried out in order to study the influence of different pollen conservation conditions on its main characteristics. Colour, pH, acidity, humidity, microbiological load (total aerobic mesophilic bacteria, lactic acid bacteria, yeast and moulds) total polyphenols and antioxidant capacity were the parameters evaluated in this work. The effects of drying, freezing and vacuum processes were evaluated. Assays were carried out after two weeks of sample collection and changes were evaluated six moths after treatments.

Results showed that all treatments yielded a high reduction in the initial quantity of microorganisms. Dry pollen showed a higher increase of colour and polyphenols content than the other treatments. In general, the antioxidant capacity of the product was similar or a little higher for all of them.

**INSECT AND MITE PESTS INFESTING AND CONTAMINATING BEE BREAD AND POLLEN LOADS**

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Pollen loads and bee bread are compounds of proteins, carbohydrates, lipids, minerals, vitamins, various organic acids, enzymes and other substances. As valuable food for bees and very useful attractive human nutriments and raw materials for food, farmaceutical and cosmetic industries, they should be free of pests and contaminations.
The aim of this study was recognition of species composition and numerousness of acaro-entomofauna infesting hive products in question and broadening of knowledge on this topic.

Two methods were used, i.e. sifting method with use of the strainers (for pollen loads collected from pollen traps) and exhauster (vacuum cleaner) method for separation and collecting of arthropods from stored honeycombs full of bee bread. Microscopical analyses of 655 collected samples show that total over 90% was infested and contaminated with arthropods (including dead bees). Intensity of infestation was diversified from single to hundreds of pest specimens per 100 g of sample. Stored bee bread was usually stronger infested than fresh pollen pellets taken from pollen traps. List of insects and mites includes over 40 identified species. Among them were mainly synanthropic stored product pests associated also with beehives, i.e., beetles, moths and psocids: Tribolium madens, Dermestes lardarius, Dermestes maculatus, Achroia grisella, Galleria mellonella, Lachesilla pedicularia, Liposceslis divinatorius. Among mites were indentified mainly acaroids, commonly known stored product species belonging to Acaridae, Carpoglyphidae and Glycyphagidae families (e.g., Carpoglyphus lactis, Glycyphagus domesticus, Tyrophagus longior); parasites (dead Varroa) and predators (Aceosejidae, Cheyletidae) were also observed.
The main issues in HONEY analysis at present regard general quality and authenticity. Control of honey quality is carried out according to the standards of the Codex Alimentarius and the EU using the harmonised methods of the IHC. Residues is another important issue of honey quality. There are different contamination risks, outlined recently the main problem at present being antibiotic residues (Bogdanov, Apidologie, 38, 2006). Authenticity includes 3 aspects: production, geographical and botanical origin. Honey adulteration is tested most efficiently by stable isotope analysis. The geographical origin is tested mostly by pollen analysis. The authenticity of botanical origin is examined by two methods: The classical approach by combined assessment of sensory, microscopic and chemical results. New methods like analysis of volatiles and spectroscopy are also very promising for the future. Control of BEESWAX is generally carried out according to the Pharmacopoe, but for wax adulteration gas-chromatographic methods should be used. There are no international standards for the regulation of PROPOLIS, POLLEN and ROYAL JELLY control. Drafts for propolis, pollen and royal jelly standards have been proposed by the IHC. The propolis draft includes proposals for the two main types of propolis present on the market, originating from poplar and Baccharis. They include general criteria like humidity, wax content, mechanical impurities, and also the main biologically active compounds, the polyphenols. The pollen draft for includes humidity, lipids, proteins, as well as biologically active compounds like vitamins and polyphenols. The royal jelly draft includes humidity, protein, carbohydrates, lipids (mainly 10-hydroxydecenoic acid), minerals and acidity. As these bee products are often used for health enhancement purposes, their contaminant level should correspond to the Pharmacopoe requirements.
Especially in Germany quite good prices for unifloral honeys from special regions can be fetched. Due to cover their expenses beekeepers within the EC have to rely on higher prices for their bee products. But also beekeepers form overseas selling good bee products without residues should participate of better prices which can be achieved for those products in the EC. But to guarantee the indicated origin to the consumer quality insurance and quality control it is of great importance.

In this field pollen analysis of honey, melissopalynology, is of great importance. Honey always includes numerous pollen grains (mainly from the plant species foraged by bees) and honeydew elements (like wax tubes, algae and fungal spores) that altogether provide a good ‘fingerprint’ of the environment where the honey comes from. Due to the fact that pollen of different plant species can be distinguished and flora of different regions varies pollen analysis can be useful to determine and control the geographical and botanical origin of honeys.

In case of looking for botanical origin it is also necessary to carry out sensory and physico-chemical analyses beside pollen analysis. The main criteria are sensory, consistency, colour, sugar spectra and electrical conductivity. In some cases also the amount of proteins and special aromatic components can be helpful. At present some new methods like infrared-spectroscopy are evolved and checked for meaningfulness. Bee colonies never will produce honey from only one source. So beside the main honey flow you always will have some other parts of nectar and honeydew sources in honey. The directive for honey (2001/110/EC) takes this into account. Unifloral honeys should come wholly or mainly from the indicated source. In Germany mainly was defined in the commentary of food law at least 60 %. The EC commission has pointed out that mainly is close to wholly. Other questions which can be settled in this field e.g. can blends of honeys be indicated as unifloral honeys.

The geographical origin can be distinguished by pollen analysis. But there is a problem with filtered honeys. After filtering there are no pollen grains left. At present isotope analysis are evolved. Current analysis on isotopes proportion (d13C/12C, d18O/16O, d2H/1H) shows encouraging results.

**IDENTIFICATION OF UNIFLORAL HONEYS BY FOURIER-TRANSFORMED INFRARED SPECTROSCOPY**

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Honey is a product with a wide range of natural compounds depending on the floral source. Pollen analysis, sensorial testing and physiochemical characterisation of a honey sample are the parts of the authentication. In order to increase sample size and reduce costs of analysis, there have been many efforts to find alternative methods. In general, the infrared spectroscopy is a useful tool in food analysis. The FTIR instrument used for our study is constructed for routine measurements and has been established for analysing most of the physiochemical parameters of honey: sugar compounds, pH, electrical conductivity and free acidity can be measured with a high reliability, for HMF and proline it is used as a screening method.
In addition, FTIR can be used for determination of the botanical origin of a honey sample by comparison of the complete mid-infrared spectra. After calibration the floral source and the physiochemical parameters can be analysed in parallel.

Honey samples from local beekeepers were collected and characterized by standard methods. The most common honey types were used for calibration.

We recorded the complete mid infrared spectrum from each honey sample. PCA-calibration models were used for comparison of the spectra. Validation was performed with samples of various botanical origins.

Honey of rape, false acacia, heather, clover, lime tree, honeydew, sunflower and cornflower was calibrated and improved during routine analysis. Most of the honey samples from rape, false acacia, heather and honeydew can be classified correctly by the FTIR in consideration of the physiochemical and sensorial properties.

**DETERMINATION OF BOTANICAL AND GEOGRAPHICAL HONEY ORIGIN BY ELECTRONIC NOSE**

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Determination of honey botanical origin is essential factor that is required prior entering any market, and it is factor that dictates price. Problems associated with botanical origin determination are rooted in fact that required analyses (pollen analysis, physicochemical parameters determination and sensory analysis) are arduous and time consuming.

This work examines performance of electronic nose based on 12 MOS and 10 MOSFET sensors in classification of different honey samples collected in Croatia with respect to their botanical and geographical origin. Data analysis is performed with StatSoft Statistica 7 software package using modules for PCA (NIPALS algorithm) and ANN. Classification results clearly demonstrates ability of electronic nose to distinguish different types of honey, and within same honey type differences that can be correlated with geographical origin.

**QUALITY EVALUATION AND APPLICATION OF RESPONSE SURFACE METHODOLOGY TO OPTIMISE THE PH, TEMPERATURE AND TIME TO ESTABLISH THE QUALITY ATTRIBUTES OF HYDROXYMETHYLFURFURAL AND DIASTATIC ACTIVITY IN EUCALYPTUS HONEY**

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In this study samples of Eucalyptus honey (Eucalyptus lanceolatus) were botanically characterized by pollen analysis. Analysis of samples for moisture, pH, hydroxymethylfurfural, diastase activity, optical rotation, mineral content and sugar composition showed that the honey samples met all the national and international standards. Response surface methodology was used to analyse the effect of temperature, time and pH on the quality responses (hydroxymethylfurfural concentration and diastatic activity) of Eucalyptus honey. A rotatable central-composite design was used to develop models for the responses. Hydroxymethylfurfural concentration was increased with increase in temperature and pH while keeping the other variables constant. Diastatic activity was decreased as the pH moved away from the optimum value of 5.2 to 5.6. Three-dimensional response surfaces were superimposed, and the regions meeting the diastatic activity calculated as diastase number (9 to 23.09°G) and Hydroxymethylfurfural concentration (3 to 10.21 mg/kg) was identified at 48±1°C for 9.5±1 minutes at 5.1±0.15 pH. These predicted values for optimum process conditions were in good agreement with experimental data.

STUDY ON SULFONAMIDE RESIDUES IN HONEY

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Honey as foodstuff of animal origin is subject to constant monitoring for the presence of banned substances or for the excessive contents of substances which can be used in animals and which are eventually allowed to be present in specified amounts. In order for a given honey to be marketed the results of the control tests must agree with the provisions of the EC regulation on the establishment of maximum residue limits (MRL) of veterinary medicinal products in foodstuffs of animal origin. In the above regulation there are no residue limits for antibacterial substances (antibiotics and sulfonamides) in honey. Hence the finding of any amount of above-mentioned substances may provide the basis to disqualify this product even though the amounts of the detected contaminants are varied and the results are directly affected by the analytical methods used. Beekeepers in the Union countries have not ceased to administer drugs to control American and European foulbrood, in spite of the prohibition of their use. Research conducted in Poland also indicates that domestic honey is contaminated mainly with sulfonamide residues. It bears out the most frequent use of the sulfonamide drug – Polisulfamid (sulfathiazole, sulfacetamide, sulfamethazine) – in Polish apiaries.

The aim of our the study was to optimize the procedure for sulfonamide residues analyses in honey and their control in commercial honey. Separation, identification and quantity analysis of sulfonamides in honey were carried out using SHIMADZU HPLC instrument equipped with fluorescence detector after synthesis of sulfonamide derivatives with fluorescamine. Chromatographic conditions were performed using analytical standards of sulfathiazol, sulfacetamide, sulfamethazine and p-aminobenzoic acid (PABA), naturally present in some honeys. With these conditions p-aminobenzoic acid (PABA) was well separate from sulfamethazine. In a practical analysis limit of detection was set at 5 µg/kg for each of three analysed sulfonamides. Received results show that sulfonamide residues in commercial honey ranged from slight up to above 108
1000 µg/kg. To take into consideration practical detection limit (5µg/kg) sulfatiazole and sulfamethazine were detected in 50% of commercial honey samples, sulfacetamide in 16%. Total sulfonamides level in 30% of honey samples was above 50 µg/kg – quantification limit establish by Polish National Monitoring Program 2005.

CHROMATOGRAPHIC DETERMINATION OF ARTEPILLIN C IN BRAZILIAN PROPOLIS

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Propolis is a resin of varied color and consistency collected by honeybee, Apis Mellifera, from several parts of the plant such as sprouts, flower buds and resinous exsudatos, being transported to the hive with the intent of defending it. Studies performed with the Brazilian propolis showed the presence of several phenolic compounds, as the most recent brings the identification of prenyl derivative of coumaric acid. Among them, the most studied one is 3,5-diprenyl-4-hydroxycinnamic acid (Artepillon-C), isolated from the propolis produced in areas which flora is rich in Baccharis species. Its quantification has become an important factor as indicator of Brazilian propolis quality. This phenolic acid has biological properties such as antitumoral, antibacterial and antioxidant activity showing that Artepillon C can be one of the most important active compounds from Brazilian propolis. It is used as a chemical marker for the quality control and exportation of green propolis. The purpose of this work is to validate the method and evaluate the content of Artepillon C from 33 samples collected in different Brazilian regions. The method used to quantify Artepillon C is an HPLC method using RP-column (C-18) at 25oC, mobile phase with acetonitrile / phosphoric acid (0,1%) in a proportion of 50: 50 (v/v), UV-VIS detection at 280 nm and retention time at 15 min. The validation parameters studied were: linearity, accuracy, precision, quantification limit and detection limit. The results obtained for the validation parameters were: detection limit = 0.0036 ug/mL, quantification limit = 0.012 ug/mL, accuracy = 0.0064 (SD) and 0.078 (RSD), recovery of 98 to 102%. Artepillon C content varied from 0 to 11% depending on the geographical origin. The southeast region of Brazil presented the highest level of Artepillon C (5.0 – 11.0%) while the northeast region did not show any Artepillon-C.

ANTIBACTERIAL ACTIVITY OF PROPOLIS SAMPLES FROM DIFFERENT GEOGRAPHICAL REGIONS OF TURKEY AGAINST TWO FOOD-BORNE PATHOGENS, SALMONELLA ENTERITIDIS AND LISTERIA MONOCYTOGENES

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Antibacterial activities of 25 propolis samples collected from different regions of Turkey were investigated against two food-borne pathogens, Salmonella enteritidis
(ATCC 13076, SE) and Listeria monocytogenes (ATCC 1462, LM). Ethanol extracts of the propolis samples (EEPs) were prepared and chemical compositions EEPs were determined by GC-MS. Antibacterial activities of EEPs were tested at concentrations of 10% and 1%, and evaluated according to the reductions in the viable cell number of the bacteria by using pour-plate method. All EEPs were bactericidal for both bacteria at concentration of 10%. Totally 19 EEPs had inhibitory effect on LM at 1%. Eight EEPs at this concentration completely inhibited the growth of LM whereas 11 EEPs caused 0.60-5.93 log reductions in the viable cell numbers. Fifteen EEPs had no effect against SE at 1%, other 10 EEPs had only limited effect with 0.04-0.71 log reductions in the viable cell numbers. Seven main organic compound groups were determined in EEPs; flavonoids, aromatic acid esters, aromatic alcohols, aromatic acids, aliphatic carboxylic acids, terpenes, and aliphatic carboxylic acid esters. Flavonoids were the only shared component found in the EEPs, which were active on LM at 1%. However, flavonoids as well as aromatic acid esters were not detected in the EEPs, which were not active on LM at 1%. Chrysin, 4\',5-dihydroxy-7-methoxyflavanone and 3,4\',7-tri methoxyflavanone were the flavonoids detected in EEPs. Chrysin was detected in 12 EEPs out of 19, which were active on LM but not detected in the other 6 EEPs, which were not active on LM at 1%. The results of the study indicate that antibacterial activity of propolis is mainly depended on its source, chemical composition and the concentration of active compounds.

DETERMINATION OF TOTAL FLAVONOID CONTENTS IN PROPOLIS SAMPLES COLLECTED FROM EAST-ANATOLIA-TURKEY

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Thirty propolis samples collected from 10 bee-farms located in the region of Kemaliye-Erzincan at east-Anatolia-Turkey were analyzed by UV-Visible Spectrophotometer and total content of flavonoids were determined after extracted into ethyl alcohol in saturated solutions. In this study, propolis samples were collected from every research beehive either in spring and summer times.

In this study, UV-Visible Spectrophotometer was used to determine the total flavonoid contents in the propolis samples measuring the adsorption peak areas. In UV-Visible Spectrophotometer, absorption peak in between 275-315 nm was characterize the total flavonoids in propolis samples. From the UV-Visible Spectrophotometric results, absorption areas representing total flavonoid contents were found to be between 237571500-2952480.

The highest adsorption area was obtained for the propolis sample collected from the 7th bee-farm (summer time collected sample), and the soluble organic components and flavonoid contents given absorption band in between 275-315 nm were found maximum. For this propolis sample, absorption peak area in between 275-315 nm was measured as 2375715000 absorption area. Around the 7th bee-farm, the population of Asteraceae, Fabaceae and Scrophulariaceae plants was very high. As a result, high flavonoid content was found to be highest in this propolis sample. The propolis sample collected from second bee-farm in summer time showed the lowest absorption peak area.
In the same wavelength range. Around this bee-farm again, the same plants were found to be grown. But in this case, propolis sample was collected in summer time and we think that flavonoids are dominant in the early stage of the plants in spring time. The second highest flavonoid content was found in the propolis sample collected around 9th bee-farm and around this farm, the population of Salicaceae was very high. The dominant plant populations around the propolis samples having highest and lowest flavonoid contents (7th and 2nd bee-farms) were not different. But, the flavonoid content differences were resulted from the secondary plants and minor population type plants and also from the seasonal changes.

**RHEOLOGICAL PROPERTIES OF SELECTED CROATIAN HONEYS**

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Studies on rheological behaviour of honey, like other fluid foods, could be important for applications related to handling, storage, processing, quality control, and sensory analysis of foods. The effect of temperature on rheological properties also needs to be documented because a wide range of temperatures encountered during processing and storage of liquid foods.

In this work, the rheological properties of four nectar varieties of Croatian honeys, among them three monofloral varieties (acacia, chestnut, sage) and meadow as polyfloral honey were investigated over a temperature range of 5 to 40 °C. The honeys had water content of 13.9 - 18.4 %. The rheological measurements of honey samples were carried out on rotational viscometer. The obtained results showed that all honeys exhibited Newtonian behaviour with viscosity reducing as the temperature was increased. Their viscosity varied according to the kind of honey and the temperature of measurement. The temperature dependence of viscosity was described using the Arrhenius equation. The activation energy ranged from 95.8 - 119.71 kJ/mol.

**ACTUAL SITUATION IN QUALITY OF HONEY IN SLOVAKIA**

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Slovak centre of agricultural research Nitra, State veterinary and food institute Dolny Kubin

The quality and health safety of food are the complex of their characteristic properties defined in the legislative. Optimal level of the quality is often understood by consumers differently. The purpose of our works is to participate in protection of public health, as well as to study and to test substances which influence the biological value of food. Presented analyses don’t include all important parameters of quality and health safety of animal food of origin, but they compare achieved results from laboratory testing of selected Slovak and imported honey.

The quality of honey is affected by many different factors. They are removed from the honey bee colony until the honey is ready for human consumption.
ANTIOXIDANT CAPACITY AND PHENOLIC CONTENT OF VENEZUELAN HONEYS PARTICIPATING IN A HONEY CONTEST, 2005

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The presence of adulterated honey in the Venezuelan market is not preventing its traditional use as a medicinal product of the hive. An effort to promote a local concern for honey quality control, was done last year, but only nine honey samples were received. For this reason, additional analysis were carried out to evaluate bioactive properties such as the phenolic content and the antioxidant capacity of honey from Anzoátegui, Barinas, Bolívar, Cojedes, Lara, Miranda and Trujillo. The total antioxidant activity (TAA) was measured as µM Trolox equivalents, and the phenolic content was measured as mg gallic acid equivalents (GAE)/kg honey, compared with artificial honey made with fructose, glucose, maltose, sucrose and water. The TAA varied between 35.66-203.21 µM Trolox equivalents and the phenolic content between 52.00-182.10 mg GAE/kg honey. Although the artificial honey control showed the lowest values for both measurements, of 17.39 µM Trolox equivalents of TAA and 23.59 mg GAE/kg honey, the correlation between TAA and phenolic content of all honeys was low (r=0.52). Therefore, the antioxidant activity of this set of honeys is only partially explained by its phenolic content.

HG AND PB DETECTION OF BRASSICA NAPUS L. BEE POLLEN FROM THE VENEZUELAN ANDES

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Twenty years ago beekeepers of the Venezuelan Andes in Mérida State were honey producers, but now pollen has become more rewarding because nectar flow has decreased. Brassica napus L. is abundant in the mountains and also the most frequently collected bee pollen, more than 50% of the bee pollen collected in Misintá Páramo along year 2005 was from B. napus. In this work, the concentrations of Pb and Hg were evaluated in fresh bee pollen of B. napus. The Pb analysis was done by electrothermal atomic absorption spectroscopy (ETAAS). The Hg analysis was done by flame atomic absorption spectroscopy (FAAS). A concentration of 268.31 ± 0.0008 mg Pb/kg bee pollen was found in fresh bee pollen of B. napus The method for the analysis of lead was validated by recovery studies, a value of 101.0 ± 4.8 % was obtained for lead. A concentration of 146.15 ± 0.0012 mg Hg/kg bee pollen was found in fresh bee pollen of B. napus. The method for the analysis of mercury was validated by recovery studies, a value of 96.0 ± 2.0 % was obtained for lead. For both measurements, the relative standard deviation (RSD%) was lower than 5%, indicating that the methods were exact, precise and free of interference. The concentration of the two heavy metals evaluated
MELISOPALYNOLOGICAL ANALYSIS AND ENDANGERMENT OF FOREST PLANT ASSOCIATIONS

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With the diversity of plant species, The Republic of Croatia belongs to the richest countries in Europe. Known Croatian flora comprehends 8582 species; from that number 4266 are higher plant species dominantly from forest ecosystems. From known plant species 5.65% are endemic. Diverse climatic zones, which are characterized by three climatic types, continental, submediterranean and Mediterranean, contribute the most of all to the high biological diversity. In the Republic of Croatia forests cover 44% of land area and with forest lands, represent unique forest area on 2.485,611 ha. According to its structure, they are almost all natural. From the forest species, the most common are deciduous, with share of 84% in total timber stock, while conifer species are present on 16% of the total area. Forests are habitat for numerous honey producing plants, therefore in Croatia almost 70% of honey is produced from forest plants, from continental and Mediterranean area.

Share of forest produced honey is 31000 t with value of 34 million €, with growing demand on market. Specially important is honeydew honey from fir tree (Abies alba) and oak tree (Quercus sp.). Forest, due to specific structure and FSC management, are not loaded with pollution, honey from such areas has exceptional value.

Besides honey production, honeybees provide survival of numerous forest species, thus more hives can be found in forests.

ORGANIC HONEY PRODUCTION AND SOME QUALITY PARAMETERS IN HUNGARY

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The Carpathian basin is a good source of the tasty liquid Robinia honey. Hungary has some 200 thousand hectare of black-locust (Robinia pseudoacacia L.) forest. This gives the environmental basis for organic beekeeping as well. In the recent years the number of organic apiaries has increased though their group is still low compared to the conventional colleagues. Besides their relatively small group their product can be the leader of the very best quality honey on the market, therefore some basic components of these Hungarian honeys were investigated.

37 organic apiaries were selected from different parts of Hungary in 2004.

Honey samples (Robinia pseudoacacia, Asclepias syriaca and multifloral) were collected and analysed with HPLC to compare the fructose/glucose ratio, HMF content, and with classical analytical methods invertase, diastase activity and water content. The
degree of crystallisation was calculated through visual and taste characteristics. Honeys originated from Black locust (Robinia pseudoacacia) showed the lowest deviation in F/G ratio, however other values may have been influenced by site specific and technological factors as well.

The studied Hungarian honeys showed 69.78% invert sugar in average. The average of fructose content was 41.61%, while the glucose detected 28.17%. The mean of HMF founded 5.67 ppm and the diastase activity was 17° Gothe.

**INFLUENCE OF THE METEOROLOGY IN THE BOTANICAL CHARACTERISTICS OF HONEY**

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Honey is a natural product directly derived from the territory. The biogeographical parameters (floral patterns, edafology, meteorology, etc.) are the main responsible for the characteristics that the honey will be present.

Our work group has a database with the pollen spectra of near 1500 honey sample from Galicia, produced between 1989 and 2005. The study of the pollen content indicates that the plants of main apicultural interest are: Castanea sativa, Eucalyptus globulus and Rubus sp.. A less importance have some like: Erica sp. (E. australis, E. umbellata, E. arborea), Trifolium, t. Campanula, etc..

The pollen of these plants appears in a common way in all the studied honeys. However, taking into account the interannual variability, important differences exist in the relative proportions of each one of them. Meteorology can be the principal cause of this variation due to there isn’t important differences in bee management.

With the purpose of to know if there is a pattern of the pollen spectra of honey related to the main bioclimatic index. We have calculated the principal bioclimatic index and have correlated them with the pollen spectra of the honey.

**QUANTITATIVE CHARACTERISTIC OF NATURAL MICROFLORA OF FRESH POLLEN LOAD**

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The research has shown high natural level of microbe semination as a property of pollen load.

To determine the causes of high quantity of mold fungi and coliform bacteria in lots of pollen load (Khismatullin Rail G. and others, 2003) we conducted microbiological research of the samples gathered during May, June and July, 2005 in special nature reserve. Inoculation of the pollen load gathered by bees at forenoon was carried out next morning at 2-4°C. We analyzed 12 assays from 3 bee families and 36 samples of pollen load of different colors. General number of aerobic microorganisms was in the range of 6,6*103-1,5*107, moulds –3,0*103-6,6*105, yeast – up 7,5*105 CFU/g. Coliform bacteria (mostly Enterobacter ssp. and Klebsiella oxidaca) are educed from 31 samples, E. coli - from 2 samples. Number of enterobacteria was 103-105
CFU/g. Lot by lot there was a growth of aerobic microorganisms and yeasts in number averaged for once selected assays of pollen load.

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E. coli and coliform bacteria Citrobacter and Enterobacter cloacae prevailed in all samples gathered in rural zones in summer 2004. Considering the data of common species of enterobacteria in commercial pollen load (Khismatullin Rail G. and others, 2005) we suppose the prevailing bacteria to be a result of influence of live-stock farming.

TEMPORAL CHANGES IN HONEY COMPOSITION

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Honey samples harvested between years 1962 and 2003 were analyzed for the amounts of humidity, saccharose, glucose, fructose, HMF, proline and diastase. The most important parameter for evaluation of honey aging is HMF. (Project VEGA 1/34/75/06)

HONEY DISCRIMINATION OF THREE PORTUGUESE BEEKEEPERS ASSOCIATIONS BY PHENOLIC PATTERN

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Phenolic compounds (phenolic acids and flavonoids) in honey are known to have healthpromoting effects as antioxidants and anticarcinogens. These compounds, considered as the phytochemical profile, have also been used as biochemical markers for the honey geographic origin. Furthermore, these components make important contributions to organoleptic properties and to its physical and chemical properties.

This study involved honey samples of three nearby Beekeepers Associations of the Portuguese Trás-os-Montes region. The objective of this work was to improve the honey’s composition knowledge, with intention to know the differences between the honeys of Beekeepers Associations of this region and to gather information that might add economic value to this region’s honey.

The honey phenolic acids and flavonoids were extracted with Amberlite XAD-2 resin. Identification as well as quantification of these compounds was carried out via high performance liquid chromatography with Diode-Array detector.
The global phenolic pattern of honey contains protocatequic, p-hydroxibenzoic, caffeic, chlorogenic, vanilic, p-cumaric, elagic, and cinamic acids as well the naringenin, kaempferol, apigenin, pinocenbrin and crisin flavonoids. Using multivariate analysis, it is possible the honey discrimination of three Portuguese Beekeepers Associations by phenolic pattern.

**EVALUATION OF ANTIBIOTICS RESIDUES LEVELS IN PORTUGUESE HONEY: A CONCERTED STUDY WITH THE PORTUGUESE BEEKEEPERS ASSOCIATIONS**

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The antibiotics residual presence in honey is a current problem with negative implications, mainly commercial, since according to European legislation, antibiotic occurrence in honey samples is forbidden. In Portugal, there is a growing concern among Beekeepers with the residues of these products in honey and they are committed to producing and selling a clean health and natural product.

In order to overcome this concern, the Portuguese National Beekeepers Federation, the Beekeepers Associations and Bragança’s Agrarian Superior School participate in a project which aims to trace antibiotics in the honey and study the different ways of honey contamination by these residues (project financed by the Portuguese National Apicultural Program). The main objective is to trace the antibiotic residues to national level in order to infer the global situation of the Portuguese honey and to promote its quality; the second objective meets the necessities of the beekeeper of identifying sources of honey contamination.

The Beekeepers Associations send a number of samples proportional to the volume of honey production, with preference being given to the commercial honey with its own label. Different classes of antibiotics in honey were analyzed such as sulphonamides, tetracyclines and Streptomycin. The screenings were done by CHARM II and the positive results were confirmed by HPLC with fluorescence or UV detector. In the case of the positive results, inquires were sent to the Beekeeper Association so that a survey could be carried out, with the apiculture involved, for evaluation of the contamination origin.

The results show that clearly there are no residues of Streptomycin, while tetracyclines were found in only one sample. The sulphonamides are the main concern but, as the results are low, they reflect external or previous years contaminations.
ANTIOXIDANT ACTIVITY OF BEE-COLLECTED POLLEN FROM *PAPAVER SOMNIFERUM* L. AND THEIR NUTRITIONAL VALUE

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Bee-collected pollen is an apicultural product which is composed of nutritionally valuable substances and contains considerable amounts substances, which may act as potent antioxidants. Carbohydrates are the principal components. Pollen is known to hold a wide variety of nutrients, including vitamins, minerals, amino acids, plant hormones, antioxidant factors, enzymes, phytosterols and more. The aim of the study was to measure antioxidant activity of bee-collected pollen samples from *Papaver somniferum* L. and analyse their chemical composition. There were selected components in pollen: proteins, various amino acids, fructose, and glucose. Absorbance at 470 nm was measured at different time intervals using spectrophotometer. Consequently antioxidant activity was calculated. Antioxidant activity of pollen from *Papaver somniferum* L. was 63.75 ± 0.79 %. It was estimated also content of proteins in pollen (250.2 g.kg⁻¹). The amino acid content was - arginine 11.7 g.kg⁻¹, glycine 9.8 g.kg⁻¹, histidine 7.1 g.kg⁻¹, isoleucine 10.1 g.kg⁻¹, aspartic acid 18.7 g.kg⁻¹, glutamic acid 19.8 g.kg⁻¹, leucine 16.7 g.kg⁻¹, lyzine 8.0 g.kg⁻¹, tyrosine 6.0 g.kg⁻¹, alanine 10.4 g.kg⁻¹, valine 12.4 g.kg⁻¹. The content of fructose was 188.5 g.kg⁻¹ and glucose 152.2 g.kg⁻¹. This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-026704.

PRESENCE OF ANTIBIOTICS AND SULFONAMIDES IN HONEY AND ROYAL JELLY ON THE EUROPEAN MARKET

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In 2005 in 5 European countries (Belgium, Great-Britain, Italy, Portugal and Spain) commercial honey samples were bought by different consumer’s organisations and screened for residues of streptomycins, tetracyclines, macrolides and chloramphenicol by means of accredited receptor and immunological tests. The screening of sulfonamides was performed with LC/MS-MS. Samples screened as positive were sent to an external lab for physicochemical confirmation by LC or LC-MS. In 20.0 % of the honey samples at least one residue (streptomycin, tetracycline, tylosin, sulfathiazole and/or sulfamethazine) was present. All honey samples were free from chloramphenicol at the level of 0.1 µg/kg. For the 5 countries involved in the study, the following respective percentages of positive samples were obtained: 19.0% (BE, 4 out of 21), 15.0% (GB, 3 out of 20), 31.6% (IT, 6 out of 19), 35.0% (PT, 7 out of 20) and 0% (ES, 0 out of 20).

In 12 out of 23 royal jelly samples bought in Italy, Portugal or Spain high levels of chloramphenicol were found. The 4 samples of royal jelly bought in Belgium were free from residues of chloramphenicol. There was no sampling in Great-Britain.
A SURVEY ON PORTUGUESE HONEY PHYSICO-CHEMICAL PARAMETERS

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There is a growing demand of natural products in human diet, both due to the possible negative effects of synthetic food additives on human health and to the increased consumer perception of these products benefits. Among them we find honey, one of the most complex foodstuffs produced by nature and certainly the only sweetening agent that can be used by humans without processing. Honey characteristics are directly dependent on the flora visit by bees, and this is reflected on its composition. Physico-chemical analysis is used routinely to classify different types of honeys and evaluate their quality. With this work we present a general idea on the chemical properties of Portuguese honeys and contribute to their classification.

Honey samples were directly obtained from the beekeepers and collected at different locations across the country, including islands, with help of the National Federation of Portuguese Beekeepers and their partners. Standard parameters as colour, water content, pH, free acids, lactones, electrical conductivity and sugars, together with total phenols content, were collected using the methods validated by the International Honey Commission. Multivariate analysis helps to look at the sample in its entirety and not just at a single component, if we wish to untangle all the complicated interactions between the matrix constituents.

The results allowed us to associate parameters values to specific regions in Portugal, simply because there are differences in botanical origin: higher values of electrical conductivity are found for darker honeys and higher levels of total phenols and can be located mostly on the north/centre regions. South shows higher amounts of light honey with lower electrical conductivity.

DETERMINATION OF SULPHA DRUGS IN HONEY BY LC-MS/MS

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Sulpha drugs are sometimes used in apiculture to prevent or to treat bee diseases as nosemosis or bacterial brood diseases. This practice, causing residues in the honey is illegal in Europe since no MRLs for sulphonamides are fixed for honey (Council Regulation N°2377/90). In case of analysis, screening is done making use of the Charm II-Sulpha Honey test. For the confirmation of the results, a liquid chromatographic-tandem mass spectrometric method was developed and validated according to Commission Decision 2002/657. Clean-up was based on the method described by Maudens et al. In short, an aliquot of honey was dissolved in hydrochloric acid followed by an extraction with acetonitrile and a SPE clean-up making use of C_{18} columns. The extracts were injected into the LC-MS/MS system. Sulfachoropyridazine was used as internal standard. For the 7 sulpha drugs tested in honey the decision limit (CCα) was 2 µg/kg and depending on the type of sulpha drug the detection capability (CCβ) was in the range of 2.2 – 2.4 µg/kg. All these values are far below the action limit.
of 20 µg/kg set in Belgium for the group of sulphonamides. Other validation parameters tested were linearity, specificity, recovery, repeatability and intra-reproducibility.


**POLLEN CONTENT OF SUMMER HONEYS FROM SOUTH-EASTERN POLAND**

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In the years 2001-2005 the samples of twenty nine summer honeys were collected from apiaries of different localities of south-eastern Poland. Pollen analysis of honey sediments was made according to the methods recommended by the International Commission for Bee Botany IUSB (Louveaux et al. 1978), whereas palynological examination by Zander classification (1935, 1937).

Results of microscopic studies have shown the presence of 82 pollen taxa (from 42 plant families), among which 61 represented by nectariferous and 21 by non-nectariferous – anemophilous and entomophilous plants. Non-nectariferous species pollen percentage in the honey samples hesitated between 0.6 and 66.4. A particular honey contained in total 13-41 (averaging 27) pollen types. The honeydew elements have been found in 22 samples.

The most important source of nectar for bees were the following taxa: Brassicaceae and Trifolium repens (in 100% of samples), Prunus, Rubus (93.1%), Centaurea cyanus, Salix (86.2%), Tilia, Anthriscus (82.8%), Fagopyrum (79.3%), Taraxacum (75.9%), Frangula (72.4%). Pollen of the mentioned taxa appeared in the investigated samples as a dominant, secondary or important minor. Among the nectarless taxa the highest frequency (over 50%) reached: Poaceae, Rumex, Plantago, Ranunculus, Filipendula.

Eight of the examined honeys were unifloral: three from Fagopyrum and one by one from Cucurbita, Myosotis, Prunus, Robinia and Tilia. The remaining 21 samples were classified as multifloral honeys with high pollen contribution of Brassica napus and other Brassicaceae, Fagopyrum, Phacelia, Rubus and Trifolium repens.

**QUANTITATIVE PALYNOLOGICAL ANALYSIS OF HONEY: AN AUTOMATIC METHOD MUCH FASTER AND ACCURATE**

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Palynological analysis of honey is important not only for the determination and control of the geographical and botanical origin of honey but also for obtain further information about honey extraction and conservation. These tests consist in a quantitative and a qualitative analysis. The first gives information on the total number of figurative element present in the honey. The second achieves the taxonomical
classification and the percentage of different pollen types occurring in the samples. Both analyses are time consuming.

In this work we present a tool for the automatic quantitative palynological analysis of honey. For the preparation of the slide we use the procedure described in Von der Hoe et al., 2005. Apidologie (35) S18-S35. Slides are observed under an epifluorescence microscope. An appropriate combination of the excitation, dichromatic and emission filters allows the rapid distinction of the figurative elements present in the honey. The subsequent count of the plant elements is made by a dedicated software for image analysis named Pollen® developed from this group. We also demonstrated that this method is more faster and accurate: only few minutes are needed to count up to 5000 figurative elements.

THE BASIC CRITERIA FOR DETERMINING OF DIFFERENCES BETWEEN REAL AND ADULTERATED HONEY IN TURKEY

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Turkey has enormous beekeeping potential with over 4.393 million bee colonies and diverse floral ecotypes. In terms of honey production Turkey has been ranked 2nd in the world rank with 73,929 tone produced in 2004.

Many various honeys (mono floral and multi floral) are produced in Turkey. Also countless various plant species raised in the country. The most important honey sources are pine, citrus, sunflower, acacia, linden and compound honey.

Several different criteria are commonly used in evaluating the quality of honey in international trade. These quality criteria include sensorial characteristics (i.e., flavor, color and taste) and biochemical analysis; moisture, pH, activity of hydrogen peroxide, invert sugar, HMF content, diastase activity, melliisopollinogical analysis. In addition the extensive analysis such as prolin content, rate of K/Na, polarimetric analysis and C13 test are done for adulterated honey.

PHYSICO-CHEMICAL, COLOR CHARACTERIZATION AND APPLICATION OF ARRHENIUS KINETICS TO RHEOLOGY OF INDIAN HONEY

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In this study a total of Twenty Indian honey samples were analyzed and botanically characterized: these included sunflower (Helianthus annuus L.), mustard, toria, sarson, raya (Brassica sp), eucalyptus (Eucalyptus lanceolatus L.), berseem clover (Trifolium alexandrium L.). Unifloral honey was considered as such whenever the dominant pollen was found at over 45% of total pollen. The samples have been analyzed to determine: moisture, pH, hydroxymethylfurfural, diastase activity, optical rotation, conductivity and
color variables (L, a, b). A Brookfield LV model (LV, Spindle31) was used to measure rheological properties of honey samples. All of these honey varieties from different floral sources exhibited Newtonian behaviors. The apparent viscosity was found to decrease with the temperature and temperature dependence of viscosity was contrasted versus Arrhenius model (µ = µo e Ea /RT). The temperature effect on the viscosity followed an Arrhenius-type relationship. The activation energy ranged from 72930.40 to 87105.77 J/Kg mol.

**Honey discrimination of three Portuguese beekeepers associations by phenolic pattern**

_Luis G. Dias, Lillian Barros, Sonia Girante, Miguel VilasBoas, Leticia Estevinho_

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Phenolic compounds (phenolic acids and flavonoids) in honey are known to have healthpromoting effects as antioxidants and anticarcinogens. These compounds, considered as the phytochemical profile, have also been used as biochemical markers for the honey geographic origin. Furthermore, these components make important contributions to organoleptic properties and to its physical and chemical properties.

This study involved honey samples of three nearby Beekeepers Associations of the Portuguese Trás-os-Montes region. The objective of this work was to improve the honey’s composition knowledge, with intention to know the differences between the honeys of Beekeepers Associations of this region and to gather information that might add economic value to this region’s honey.

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**Characterization of Croatian honeys by their physico-chemical characteristics**

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Honey samples that are available commercially, differ in quality on account of various factors. These include geographical, seasonal and processing conditions, floral source, packaging and storage period. Although Croatia is a small country, there’s a wide range of different types of honey present on domestic market. In this study a total of 220 Croatian honey samples of known origin harvested in three consecutive years (2003., 2004. and 2005.) were analysed. Among honeys studied there were: 108 samples of acacia, 21 floral, 18 sage, 27 chestnut and 46 meadow. The following parameters were
determined: water, total reducing sugars, sucrose, electrical conductivity, acidity, hydroxymethylfurfural (HMF) content, diastase activity and proline content. The aim was to evaluate the quality of selected honeys according to the current legal regulations in Croatia.

**UPLC-TOF-MS – A USEFUL TOOL FOR THE ANALYSIS OF HONEY?**

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The authenticity of honey is almost exclusively characterised by the microscopic analysis of pollen which can only be carried out by trained personnel. Therefore, chemical-analytical indicators are required to determine the individual kinds of honey.

Herefore, as already published [1,2,3], phenolic acids and flavonoids are suitable substances.

Most of the phenolic acids can easily be determined by GC-MS after derivatization, but HPLC-DAD would be preferable for analysing both groups. Nevertheless, due to the quantity of the compounds in honey, their similar structures and their different UV-sensitivity, the analysis is a complicated matter.

Recently, the Ultra Performance Liquid Chromatography combined with TOF–MS (UPLC-TOF) was used by our work group. The advantages and disadvantages will be presented and discussed.

Literature:


**ULTRAFILTRATION OF HONEY–EFFECTS ON ENZYMES, CHLORAMPHENICOL AND HMF**

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Recently, the process of ultrafiltration was applied to honeys to avoid crystallization. Thereby, pollen and antibiotics as well as larger molecules like proteins can be removed [1].

The effects of ultrafiltration on the composition of honey, especially on the enzymes diastase and saccharase, on chloramphenicol and HMF will be introduced. The ultrafiltration process was carried out on the laboratory scale, and the products were analysed and compared. The results presented here show that the contents of these components are considerably influenced by ultrafiltration.

Literature:

INFLUENCE OF RIPENING HONEYS IN CONTROLLED CONDITION ON THEIR 
PHYSICOCHEMICAL PROPERTY

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Samples of honey harvested before the end of a nectar flow. Several honey combs 
with uncapped honey were extracted and dehydrated in a chamber equipped with a 
dehumidifier. Before and after the dehydrating process the samples of honey were taken 
to be analysed. Other honey combs were left in the hive and samples of ripen honey 
(conventional) were taken after the end of the nectar flow to compare the quality of 
undehydrated, dehydrated in controlled conditions and conventional honey. Water 
content, free acidity, sugar content, electrical conductivity, diastase activity were 
determined in the samples. The initial content of water in honeys was 23.06% and after 
12 hours of the dehydrating process honey humidity dropped by 1.78% on average. After 
36 hours of the dehumidification process the average content of water was 16.46%. In 
conventional honeys the average content of water was 17.91% which is higher than that 
in honey dehydrated in controlled conditions.

The reduction of excess water content caused increase of free acidity, sugar content 
and diastase activity. Diastase activity increased average by 3.49 on Schade scale. Free 
adidity content increased by 1.1 mval/kg in comparison to undehydrated honey. Sugar 
content also increased; the sum of fructose and glucose increased by 5.41% on average 
and sucrose by about 0.22%. A little higher average diastase activity and free acidity 
determined, in comparison to dehydrated honey but sugar content was lower. The 
ripening of honey in controlled conditions allows the water content in honey to decrease 
and it is not harmful to honey quality. Reduction of water content causes increase of free 
adidity, sugar content and diastase activity. The parameters of dehydrated honey do not 
differ considerably from those of conventional honeys.

POLLEN SPECTRUM AND PHYSICOCHEMICAL ATTRIBUTES OF CROATIAN 
MEDITERRANEAN MULTIFORAL HONEYS

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The aim of this work was to determine which floral sources prevail in Croatian 
Mediterranean multifloral honeys and its physicochemical characteristics. Honey (55 
samples) verified by pollen analysis as multifloral, were collected during the 2004 
harvest directly from beekeepers from different locations across Croatian Mediterranean 
area. The following determinations were carried out: moisture content, electrical 
conductivity, specific rotation, pH and acidity, hydroxymethylfurfural (HMF), diastase 
and invertase activity, proline content and sugar composition.

Pollen analysis of honey samples showed a wide variety of botanical sources. In a 
melissopalynological study of investigated honey samples, 78 pollen types were
identified. Samples ranged from 8 to 21 different plant species. The principal species identified were: Paliurus spina christi, Fraxinus ornus and Trifolium pratense gr.

In accordance with a variety of nectar sources, samples have relatively wide range of physicochemical parameters: electrical conductivity (0.14 mS/cm - 0.87 mS/cm), pH (3.74 - 5.12) and free acidity (11.3 meq/kg - 37.1 meq/kg), specific rotation ((-15.4) - (-4.1)), proline content (234 mg/kg - 960 mg/kg), invertase (6.2 U/kg - 405.8 U/kg) and diastase activity (Schade scale) (14.1 - 57.7).

Though samples are diverse, all together they have high enzymatic activity which is followed by low HMF content.

SCREENING OF THE POTENTIAL PRESENCE OF PYRROLIZIDINE ALKALOIDS IN HONEYS BY SPECTROPHOTOMETRIC DETERMINATION

R.A. Pérez Martín, M. González Lorente, P. Lorenzo Lozano, and C. de Lorenzo Carretero


Pyrrolizidine alkaloids (1,2-dehydro-pyrrolizidine ester alkaloids, PAs) are a large group of natural toxic products from the secondary metabolism of some plants. Plants containing these alkaloids are found in representatives of the families Fabaceae (Leguminoseae), Boraginaceae and Asteraceae (Compositae). The genus Echium (Boraginaceae) is widely distributed across Europe and their species Echium plantagineum L. and Echium vulgare (Viper’s bugloss) are important nectar sources for honeybee. Honey research, as potential way for the APs ingest, is relatively recent and complex.

Melissopalynological analysis of 127 Spanish honeys was carried out, the results showing an important incidence of the genus Echium in these samples, thus suggesting the necessity to develop a method for the screening of the samples. With this objective, different extraction procedures were assayed using honey free of Echium palynomorphs, fortified with two PAs standards. The extractive method selected for further analysis was the SPE with Extrelut column (Merck).

Screening of honey extracts were done by means of spectrophotometric analysis of the coloured solution resulting of a first treatment of the extract, to yield a pyrrole derivative by PA’ dehydrogenation, followed by coupling with 4-dimethylaminobenzaldehyde to obtain the coloured solution. In this screening, twenty one honeys with different quantitative presence of Echium pollen were spectrophotometrically evaluated, the results yielding positive identification of alkaloids in honeys with some content of pollen from Echium in their melissopalynological analysis. The effect of the matrix colour was taken in account in these determinations.
SPECTRUM OF MELIFEROUS PLANTS EXPLOITED BY APIS MELLIFERA ADANSONII IN HIGHLIGHT SOUDANO- GUINÉENNE ZONE OF WEST CAMEROON

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Melissopalynology analyses of 30 honey samples collected between September 2002 and March 2003 in the western soudano-guinean highlands of Cameroon were carried out in order to evaluate frequency and intensity of exploitation of melliferous plants by Apis mellifera adansonii. The main result were as followed:

- Spectrum of melliferous plants gathered is wide and diverse. Indeed pollen of 88 genus of melliferous plants of 46 families were identified in honey samples. In term of number of plants genus the most represented were Asteraceae, Euphorbiaceae and Myrtaceae.

- As concern frequency of exploitation of melliferous plants, melliferous plants could be grouped into three categories: most frequently gathered melliferous plants: Eucalyptus, Coffea, Bidens and Terminalia; moderately gathered melliferous plants: Elaeis, Phoenix, Lannea and Julbernardia. Finally less frequently gathered melliferous plants with their pollen present in less than 30% of honey samples (Salix, Casuarina, Pterocarpus etc).

- When intensity of exploitation is considered, the most intensively exploited melliferous plants by Apis mellifera Adansonii represent 22.7% of genus grouped into 16 families. Pollen of 8.0% melliferous taxa gathered belonging to six families appeared as dominant in honey samples. More than two third (92.0%) of the melliferous plants appeared as accessory, secondary or rare pollen. Eucalyptus was the only melliferous plants whose pollens were altogether as dominant, accessory and rare in honey samples. From their pollinic composition, 60% of honey samples were unifloral with Eucalyptus and Helycrisum pollen dominating, against 40% which were multifloral honey.

UNIFLORAL HONEY CHARACTERIZATION ATTEMPT BASED ON QUALITY CONTROL PARAMETERS

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Unifloral honey characterization is usually based on melissopalynology, organoleptic characteristics and very specific parameters such as amino acids, flavors, minerals, and flavonoids, among others. These parameters are difficult to determine within small and local laboratories, in which only routine quality control parameters are completely standardized. The groundwork of this work has been to try to characterize five types of Spaniard monofloral honeys by determining electrical conductivity, free acid, formol number, proline content and diastase, invertase and β-glucosidase activities. 107 samples, harvested in 2001, 2002 and 2003 were analyzed: 22 eucalyptus (Eucalyptus sp.) honeys, 35 rosemary (Rosmarinus officinalis) honeys, 15 citrus blossom honeys, 5 thyme (Thymus vulgaris) honeys, 12 lavender (Lavandula angustifolia) honeys, 9 alkeria (Alkanna tinctoria) honeys.
(Citrus sp.) honeys, 33 heather (Ericaceae) honeys and 2 honeydew honeys. Depending on the color and botanical origin of the samples, the results have been different. Dark honeys (heather and honeydew) have shown the highest values for all the parameters analyzed, in contrast with light honeys (citrus and rosemary) that have shown the lowest ones. In general, when comparing heather and honeydew honeys, proline and \( \beta \)-glucosidase activities mean values have been higher in heather honeys; electrical conductivity average has been slightly higher in heather honeys, free acid mean values have been similar in both heather and honeydew honeys and formol number, diastase and invertase averages have been higher in honeydew honeys. When comparing citrus and rosemary honeys, electrical conductivity, formol number, proline, diastase and invertase and \( \beta \)-glucosidase activities mean values have been slightly lower in citrus honeys, and free acid has been similar in both citrus and rosemary honeys. Despite the fact that light honeys have not been botanically characterized with the parameters analyzed, statistical discriminant analysis has correctly classified 95.5% eucalyptus honeys, 80% heather honeys and 100% honeydew honeys, which means that with simple, rapid, and low cost routine quality control parameters, it is possible to have an idea of the botanical origin of dark honeys.

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**GLUCOSE-OXIDASE AND CATALASE ACTIVITIES OF SPANISH UNHEATED HONEYS**

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Glucose-oxidase and catalase are enzymes that have been hardly studied in honeys. Analysis of both enzymes is not simple, because there are lots of interferences due to many components of this food. However, the determination of glucose-oxidase and catalase is very important because these enzymes are directly and inversely correlated with antibacterial activity of honey, respectively. Within honey, bees’ glucose-oxidase produces hydrogen peroxide which is considered by many researchers as one of the main antibacterial honey agent. Furthermore, glucose-oxidase contributes to honey acidity due to the formation of gluconic acid what makes honey more stable against fermentation. The origin of catalase is mainly vegetal, although it can be also produced by some yeasts and other microorganisms. As catalase breaks hydrogen peroxide molecule, its activity is inversely correlated with antibacterial activity of honey. Glucose-oxidase has been determined by spectrophotometric measurement, at 400 nm of the product of the reaction between o-dianisidine, peroxidase and hydrogen peroxide obtained from the action of glucose-oxidase on glucose. Catalase determination is based on the reaction of this enzyme with an excess of hydrogen peroxide with the subsequent spectrophotometric measurement at 400 nm, of the reaction product between the reminder hydrogen peroxide, o-dianisidine and peroxidase. For the analysis of both enzymes a previous dialysis step is necessary to remove interferences. Glucose-oxidase and catalase have
been determined on 23 Spanish unheated honeys. 10 samples were Rosmarinus sp. honeys. 6 samples were Ericaceae honeys. 2 samples were Citrus sp. honeys. 2 samples were Eucalyptus sp. honeys. 2 samples were polyfloral honeys and 1 sample was honeydew honey. Mean value for glucose-oxidase was 305.7 µg H2O2/g honey/h, with values ranging from 106.5 to 718.5 µg H2O2/g honey/h. For catalase, values ranged from 1.6 to 107.6 Kf 10-3/g honey/min, with an average of 41.2 Kf 10-3/g honey/min. NOTE: The Spaniard INIA (Ministerio de Ciencia y Tecnologia, Spain) has supported this work under the project CAL01-066-C7-3.

A COMPARISON OF PINE HONEY’S VOLATILE COMPOUNDS WITH THOSE OF HONEYDEW PRODUCED FROM MARCHALINA HELLENICA

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Honey is derived from flower nectar and honeydew. Pine honey is the most common Greek honeydew honey, which is produced in autumn from honeydew secreted by the insect Marchalina helenica. This insect is restricted to Pinus brutia, Pinus halepensis, Pinus sylvestris, and Pinus pine.

In this work pine honeys samples and honeydew secretions from Marchalina helenica of the same area were collected and analysed using a Purge & Trap – GC – MS. A total of 46 and 27 volatile compounds were identified in the samples of pine honey and honeydew respectively. A great proportion of the volatile compounds in honeydew were terpenes. The substances α- and β-pinene (31.3 and 17.7%), δ-3-carene (30.9%), α-terpinolene (4.4%), camphene (4.3%) and β-phellandrene (3.2%) were found in high percentages. Fourteen of honeydew’s volatile compounds were also detected in the honey. The results of this research verified that some of the volatile compounds of tree are transferred to the honey without conversion.

THE FORMATION OF FURAN DERIVATIVES IN FIR HONEY DURING HEATING

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Honey is a concentrated solution of various sugars, which is produced by bees from the nectar and honeydew. The 5% of the annual greek honey production is fir honey. The heating process effects its quality as changes observed in moisture, sugars, amino acids, enzymes, HMF microorganisms and volatile compounds.

In the present study fir honey samples were heated in different temperatures for four period of time and the samples were analysed using a Purge & Trap – GC – MS system. Seven furan derivates (2,3-dihydro-4-methyl-furan, dihydro-methyl-3[2H]-furanone, 2-furanyl-methanol, 5-methyl-2-furanyl-carboxaldehyde, benzofuran, 2,2’-furan, 2-ethyl-5-methyl-furan) were formed during heating. Furfural and 1-(2-furanyl)-ethanone were detected in the control honey but their concentrations increased
particularly after heating at 65°C. The formation depended from the temperature and the duration of the heating.

HONEY GRADING USING FUZZY LOGIC

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Classification is very important for the evaluation of agricultural crops. Unfortunately, subjectivity, tediousness and inconsistency in grading by human experts enforce the post harvest process to apply automation in honey grading. Fuzzy logic (FL) was applied as a decision making support to grade honey in this study. Chemical features such as the mineral (%), Invert sugar (%) and Moisture content (%), Acidity (meq kg⁻¹), HMF (mg kg⁻¹), Diastase level, and Sucrose (%) were measured through different laboratory equipment and methods. The same honey samples were graded by both a human expert and a FL system designed for this purpose. Grading results obtained from FL showed 97.14 % general agreement with the results from the human expert, providing a safe check for expert’s observations in honey grading.

TRACE HEAVY METAL LEVELS IN HONEYS FROM DIFFERENT REGION OF ANATOLIA

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A survey of 25 honey samples of different botanical origin, collected all over the Turkey was carried out in order to assess their heavy metal contents. The aim of this study was to determine the levels of cadmium (Cd), lead (Pb), iron (Fe), manganese (Mn), copper (Cu), nickel (Ni), chromium (Cr), zinc (Zn), aluminum (Al) and selenium (Se) in honey samples from different region of Turkey. Heavy metal contents were determined by flame and graphite furnace atomic absorption spectrometry after microwave digestion, wet digestion and dry ashing. The accuracy of the method was corrected by standard reference material (NIST-SRM 1515 Apple leaves). The contents of trace heavy metals in honey samples were found to be in the range of 0.23-2.41 µg kg⁻¹, 0.32-4.56 µg kg⁻¹, 1.1-12.7 µg kg⁻¹, 2.1-10.2 µg kg⁻¹, 9.6-105.8 µg kg⁻¹ and 2.6-29.9 µg kg⁻¹, 2.4-37.9 µg kg⁻¹, 1.1-17.9 µg kg⁻¹, 83-325 µg kg⁻¹, 38-113 µg kg⁻¹ for Cu, Mn, Zn, Fe, Pb, Ni, Cr, Cd, Al and Se respectively. Aluminum was the most abundant of the element while iron was the least present element in Turkish honeys.
ANTIBIOTIC RESIDUES IN TURKISH PINE-HONEYDEW HONEY

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Turkey is the biggest pine honeydew honey producer and exporter in the world. 92% of world’s pine honeydew honey production is realized in Mugla Region of Turkey. The economical importance of pine honeydew honey is quite significant. Recent years, the antibiotic residue arguments are arised in pine honeydew honey from Turkey and exportation was decreased. The aim of this study was to explain the last datas (year 2005) of residue of pine honeydew honey from Turkey.

The eighty-two pine honeydew honey samples were harvested from Mugla Region of the country. Samples were analysed for the presence of residues of sulfonamide and tetracyclines in Ege University Center for Drug R&D and Pharmacokinetic Applications, in 2005. HPLC analytical method was used for the determination of antibiotic residues in honey. Residues of veterinary drugs were found in a limited number of honey samples, namely; tetracycline (1 sample of chlortetracycline, 1 sample of doxycycline, 3 samples of oxytetracyclin) 5 out of 82 (6 %) samples and sulfanamid (8 samples of sulfamethazin, 3 samples of sulfamethizol, 2 samples of sulfamethoxyprazin) 13 of 82 (16 %) samples were found. In comparing with last two years, the sharp decrease seems of the honey contamination with antibiotic residues but not the disappearence of the problem. Turkish pine honeydew honey will take a better place in the European market in near future.

SPECIFIC ROTATION AS A PARAMETER FOR NECTAR AND HONEYDEW HONEY DISCRIMINATION

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Specific rotation (SR) depends on the ratio among glucose and fructose content. Nectar types of honey have negative SR, because fructose as a prevalent sugar is laevoorotatory, while honeydew types have positive SR, because glucose as a prevalent sugar is dextrorotatory. In this way it is possible to discriminate between nectar and honeydew as the main honey sources. European Directive concerning honey differentiates nectar and honeydew honeys with electrical conductivity (EC), where 0.8 mS/cm is set as the limit value; in pure nectar honey types must be below it, and in honeydew types must be above it. Lime and chestnut honeys that are of both, nectar and honeydew origin, are exceptions in this limitation. The results of our research proved that even SR can not be used in the case of these two honeys. Nevertheless, the combination of the EC and SR is a great tool for discrimination of this two specific types of honey from all other types and mixtures, since lime honey has EC below 0.8 mS/cm and positive SR (1st quadrant), and chestnut honey has EC above 0.8 mS/cm and negative SR (3rd quadrant), respectively. For comparison, acacia and multifloral honeys
have EC below 0.8 mS/cm and negative SR (4th quadrant), and honeydew honeys have EC above 0.8 mS/cm and positive SR (2nd quadrant). The linear correlation between EC and SR for acacia, multifloral and honeydew honeys is statistically significant at the 0.01 level, it is very strong (R² = 0.96) and equal to: SR = 41.3 cm/mS * EC – 34.3. On the contrary, the correlation for lime and chestnut honey does not exist.

COLOR AND CORRELATED CHARACTERISTICS OF SLOVENIAN HONEY

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The color of five types of Slovenian honeys, namely acacia, lime, chestnut, multifloral and honeydew honey (ten samples of each) was investigated by means of two methods: color intensity (ABS450) and determination of color parameters through the CIE L*a*b* tristimulus method. In addition electrical conductivity, ash content, proteins and total phenolic content of honeys were determined and the correlation coefficients with the color were calculated.

The color of honey is related to the content of minerals, plant pigments, pollen and phenolics and is characteristic for honey floral origin. The results of this study demonstrated that the color of Slovenian honeys is very different and varies from almost colorless to dark brown. The brightest are acacia and lime honeys, almost colorless to pale yellow and white to cream or ivory, with yellow or green shade, respectively. Multiflower honeys are very colorful from yellow to brown depending on plant source and honeydew presence. The color of chestnut honey is amber, more or less dark, with reddish shade, while honeydew honeys are light to dark brown, with reddish or green shade.

Electrical conductivity and the contents of ash, proteins and total phenolics of the studied honey samples differ widely. The highest values of these parameters were obtained for chestnut and honeydew honey and the lowest for acacia honey. The correlation matrix showed a significant correlation between honey color and all other physicochemical parameters analyzed.

THE EFFECT OF THE DRYING PROCESS ON THE ANTIOXIDANTS VITAMINS FROM BEE POLLEN


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Bee-collected pollen (bee pollen) is consumed like a human dietary supplement but such as other protein and moisture rich foods, it can lose its nutritional value when stored or processed incorrectly. To avoid bee pollen deterioration it must be dried quickly and there are many preserving methods as follow: open air drying, artificial heating, solar drying and silica gel treatment. For commercial scale production, the most commonly
method used is the artificial heating to achieve moisture of 4% (established by the Brazilian regulation). It can be obtained by raising the temperature to 40-42°C. Unfortunately, in some cases, this temperature tends to diminish the vitamin content. The aim of this study is to quantify the antioxidant vitamins: C, E and beta-carotene as provitamin A in fresh and processed samples of bee pollen and to evaluate the effect of two drying processes in the vitamin content. Ten samples of fresh bee pollen were dried by either conventional method (drying at 42°C) or by an alternative method (drying at 30-35°C). Vitamin C was quantified by potentiometric titration, vitamin E by HPLC-normal phase and beta-carotene by open column chromatography. In fresh samples, vitamin content varied between 13.5 and 42.5 ug/g for vitamin E, 56.3 and 198.9 (ug/g) for beta-carotene and 273.9 and 560.3 ug/g for vitamin C. For the drying process at 42°C it was observed a loose of the vitamins as follows: 35.5 % (vitamin E), 56.5 % (provitamin A) and 8.1 % (vitamin C). For the drying process at 30-35°C it was observed a lost of the vitamins as follows: 28.9 % (vitamin E), 46.4 % (provitamin A) and 4.0 % (vitamin C). Both methods were efficient in drying the bee pollen but the alternative method, which uses the lower temperature, was more efficient in retaining the vitamins.

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**EVALUATION OF NECTAR FLOW IN NEW LOW ERUCIC ACID AND LOW GLUCOSINOLATE CONTENT LINES OF SINAPIS ALBA, BRASSICACEAE**

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Flowers of Sinapis alba show typical entomophilic features. They offer both nectar and pollen to pollinators. Sinapis alba is known as a good melliferous crop, eagerly visited by honey bees. Plants are cultivated mainly for seeds, which set highly depends on the presence of insects. After an improvement of chemical composition of seeds, S. alba can be an alternative oilseed crop, replacement for canola crops damaged by winter frosts and a source of high protein supplements. An important research objective at the IHAR, Poznań, Poland is the development of low erucic acid and low glucosinolate content lines, resulting in plants, which are devoid of sinalbin. In 2005 a study on a beekeeping value of the best 36 lines has been started. During the peak of flowering the abundance of nectar secretion as well as sugar productivity were investigated. Moreover, flowers morphology was examined. In flowers of all studied lines nectar is secreted by the median and lateral pair of nectaries, situated in the region of filament bases. However, differences were found in morphology and activity of these pairs. Usually, the lateral glands produced more nectar than the median ones, but in 7 lines the activity of both pairs was equal. In florets of 3 lines the stamens were fused, making an access to nectar difficult. Mean nectar productivity per 10 flowers differed significantly between the lines and ranged between 3.11 mg and 18.67 mg. Mean concentration of nectar was 12% - 40.3%. Ten flowers of the observed lines secreted from 0.7 mg to 4.79 mg of sugars in nectar, on average. These differences in nectar productivity and accessibility between the breeding lines can influence insect visitation on their flowers and then the yield of seeds.
Management

Symposium organized by Marco Lodesani and Vladimir Vesely

BAD BEEKEEPING MANAGEMENT TECHNICS

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Most discussions on bee management technics focus on how things should be done correctly. Though few are willing to admit it, every beekeeper makes mistakes during his beekeeping career. It is safe to say, that the longer you have kept bees, the more errors you have made. Some beekeepers (and researchers) are very skilled at making mistakes and make much bigger mistakes than others. In addition they often repeat these same mistakes. This talk considers just a few of the many management errors that I have made and illustrates why you should not do it "my way" (Unless of course, you really wish to get into real trouble).

BEEKEEPING A DEAD ART OR A LIVING SCIENCE?

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Many politicians and most bureaucrats seem to have very little idea of the important part played by bees in many aspects of agriculture. Pollination is at last getting some of the recognition it deserves in its own right but for a very long time it was seen from the stand point of the plant with little attention to the agents that accomplished the transfer of pollen.

As beekeeping does not fit easily into mainstream bureaucratic thinking it is often ignored or, even worse, forgotten. This attitude, along with the more rapid spread of hive pests and bee diseases, and an ageing beekeeping population has lead to concern for the future of all bees and beekeeping in the EU.

Without bees the world would starve and there is increasing anxiety about the need to conserve all pollinators. This paper looks back over the past 40 years and endeavours to look at the current situation as it applies to honey bees and beekeepers. It tries to offer an overview of the current trends within the beekeeping situation in Europe.
LONG-TERM MONITORING OF HONEY BEE COLONIES IN GERMANY: ORGANIZATION OF THE
PROJECT AND FIRST RESULTS FROM THE PERIOD AUTUMN 2004 – SUMMER 2006

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Losses of honey bee colonies, mainly during the winter period, are a long known threat for beekeepers. In most cases bee diseases, intensification of agriculture (including plant protection), shortage of pollen and beekeeping practice were discussed as crucial reasons. The dramatic losses of bee colonies in Europe during the winter 2002/2003 revealed that a new scientific approach was necessary to clarify the reasons for this periodical phenomenon. Therefore, we established a long-term and nationwide survey to register the regional distribution of colony losses and to evaluate the reasons mentioned above.

Our project started in autumn 2004 as a result of several common meetings with representatives of beekeeping organizations (DIB, DBIB), agricultural organization (DBV), the German Ministry of Agriculture, 9 German apicultural institutes and the chemical industry. The institutes are in charge for meanwhile 125 beekeepers with about 7,500 honey bee colonies distributed all over Germany. From 1,250 colonies (10 of each beekeeper) we receive exact data on colony development, honey yield and Varroa treatment together with various samples throughout the year. Therefore, colony losses can be correlated retrospectively with specific data such as bee diseases, residues in honey/pollen or availability of certain crops. We describe the structure of our German monitoring project and present data from the first 18 month of cooperation. The possibility of a European network on honey bee monitoring is discussed.

WHICH FACTORS ARE IMPORTANT FOR OVERWINTERING OF HONEY BEE COLONIES?

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Since 1989 the population dynamics of honey bees is investigated in a long term project. Using the "Liebefelder Methode" the development of far over 100 colonies was observed yearly to study the influence of location, weather, honey flow, Varroa infestation and beekeeping management. The overwintering is of elementary importance for the beekeeping success.

Overwintering is predominantly influenced by the colony strength in fall and the degree of Varroa infestation during the rearing of the winter bee brood in fall. During the winter the colonies normally get weaker. The mortality of the winter bees depends on the winter temperature and the location. As a rule, honey bee colonies overcome mild winters better than cold winters. They overwinter relatively bad at locations with too much wind protection and high humidity. The amount of brood cells in fall, the isolation of the hive and the bee food (sugar water or syrup) are without importance.
A good overwintering presupposes that particularly the winter bee brood is protected from excessive Varroa infestation on time. In fall and winter the damage thresholds are considerably lower than in late summer. If the number of mites is not sufficiently reduced before production of the winter bees but only at the beginning of the winter this can not avoid an earlier mortality of the winter bees. When the infestation of the winter bees was more than 10%, colonies overwinter badly or they die before springtime.

**Main Aspects of the Varroosis Control in the Czech Republic**

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Varroosis was diagnosed on the territory of the Czech Republic for the first time in the year 1981. During the passed 25 years massive mortality of bee colonies has occurred on no part of the territory of the Republic, winter losses have not exceeded the biologically justified limits and in bee products no overlimit residues have been detected.

The integrated varroosis control system is the result of the cooperation of the State Veterinary Administration, Czech Association of Beekeepers and the Bee Research Institute in Dol. System of the varroosis control includes following principles: organized implementation of areal anti-varroosis measures on the most possibly largest area, the use of medicaments with the efficacy more than 90%, areal diagnosis at every year, monitoring of the efficacy of applied medicaments.

At the present time in the Czech Republic following activities are in force: investigation of winter debris from all honey bee colonies, paint of the sealed brood before the spring at bee colonies with the find of more than 3 mites per a colony, monitoring of daily natural fall of mites in July and August, on the basis of the daily natural fall the treatment of colonies by Formidol (formic acid) or at larger intensity of the fall the treatment by strips with long term efficacy (Gabon strips).

In October all honey bee colonies are treated by fumigation (amitraz, tau-fluvalinate) and in November - December before the solstice at temperatures lower than 10° by aerosol. A week after the last treatment pads are cleaned or new pads are put on the bottom for the taking of winter debris.

Critical points of the system are as follows: the brood protection of the wintering generation of bees, radical restriction of the infection during the broodless period.

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**Professional Beekeeping in the Czech Republic**

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In the Czech Republic there are 50,000 beekeepers who own 552,000 bee colonies. The vast majority of beekeepers are hobbyists. In the last year there exist about 81 bee farms (professional beekeepers) which keep 19 thousand bee colonies, i.e. 3.4% of all bee colonies in the Czech Republic. Since the year 2002 the number of professional
beekeepers has been increased by 21 and the number of bee colonies by six thousand. We applied a questionnaire to determine the entry into European Union. Work costs for the bee colony management made 45 %, other costs on feeding, transport, maintenance, renewal of hives, material 55 %. Average yield of a colony was between 25 and 30 kg, minimal estimated economic yield moves between 35 and 40 kg per hive. Many beekeepers use small operation technology and that is the reason why they are economically not active. As a consequence of honey price reduction the economic situation has been impaired in comparison with the year 2002. The economics may bee encouraged mainly by higher honey yields and by reduction of the time necessary for the treatment of bee colonies. Most bee farms are to be provided with new equipments and transport means for moving hives, but beekeepers because of financial reason cannot afford it.
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TRANSITION TO BIOLOGICAL BEEKEEPING IN TRANSYLVANIA

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A support programme for beekeepers for eradication of residues in honey and the transition to biological methods was initiated by Apis Prod, a honey packer in Blaj (Judea Alba, Romania) in a cooperation with a consortium of Dutch partner companies, supported by the Netherlands Ministry of Economic Affairs under the PSO programme. For this a bio-association of about 90 beekeepers was formed with an affiliation to the company. An apiservice centre was set up and training on biological methods was organised on a regular basis. The beekeepers were provided with new hives with a so-called varroa drawer, bio-comb foundation and an ethereal oil mixture for treatment of varroa, to break through the established use of varroa strips containing fluvalinate and amitraz. From 2004 until 2006 most beekeepers of the association stopped using the conventional treatment and changed to bio-technical as well as bio-treatment and bio-feeding methods. Ironically sugar feeding for winter food security had just started to be adopted, but this is not allowed in biological methods. Biotechnical methods include the drone-brood method, which in the most basic form has been applied for many years by Romanian beekeepers. Sofar only one beekeeper could be certified as organic by EcolInspect. The other beekeepers are still regarded as bio-producers in transition.

RESIDUES OF ORGANIC ACIDS ARE IN CONFLICT WITH „BEST BEEKEEPING PRACTICE“

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The problem
Extensive knowledge and practical experiences with the use of organic acids (formic-, lactic- and oxalic-acid) are available for the beekeepers for the usual situation when acaricides are applied in productive colonies after the time of the active bee season or as winter treatments.
The mite population growth sometimes can reach a threshold that treatments are necessary to apply immediately before and during the active bee season. However, reliable methods and experiences in using organic acids

1. in brood less colonies (swarms, artificial swarms),
2. before and in between times of nectar flow are missing, in particular those methods that avoid residues in honey.

The methods

Taste thresholds were worked out for residues of organic acids (formic-, lactic- and oxalic-acid) in honey.

Worst case studies were implemented to evaluate possible risks after the treatment of productive bee colonies before and during the active bee season.

Organic acids were tested for the use in brood less colonies (swarms, artificial swarms) during late spring/early summer time and possible effects on the last honey crop (heather)

The results

Our results clearly show that there is a risk of residues in honey, if treatments of organic acids (formic-, lactic- and oxalic-acid) are applied in regular productive colonies before a mass nectar flow might follow. Moreover, there is no calculable safety for a reduction of the acid residues if treatments are applied in-between two periods of nectar flow.

However, treatments of brood less colonies (swarms, artificial swarms) during late spring/early summer time, which then developed to productive colonies until the last honey crop (heather), did not influenced the natural acid concentration of the harvested heather-honey.

THE APPLICATION OF GIS-TOOLBOXES IN BEEKEEPING. POTENTIAL AND PROBLEMS

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According to the definitions most often used, a Geographic Information System (GIS) is a computer based system set up by hardware, software, data, humanware and – last but not least - knowledgeware. It can be used to collect, manage, analyze and save georelated data permanently and to visualize the data or the produced maps, tables and diagrams. Based on the spatial referenced data (geodata), these GIS functionalities can be distinguished into three subsets of procedures. The input - subset deals with the data acquisition, preparation, data entry and in-system-management. The second subset (data analysis) is the central element and responsible for reviewing, pattern recognition, model building and detection of correlations and dependencies. And finally, the last subset works as a presentation module for the system’s data or the results. Each of theses modules is represented by a distinct set of tools to fulfill the GIS - specific tasks.

As well as in similar cases in environmental research the major advantage of a GIS comes into light if the spatial reference of the data plays an essential role. In that cases this kind of information system on the basis of the functionalities and structures can provide support in all spatial relevant administrative or organizational workflow sequences. Actually the system’s core consists of a database management system
containing georeferenced information (coordinates) about colonies of bees as well as associated attribute information. The analysis toolset is able to access the relational linked database tables and for a broad spectrum of different evaluation purposes. In that way the following fields of application could be supplied with ease:

- Administration and management conditions of the colonies of bees which are already (partly) regulated by different laws scoping the matters of beekeeping in connection with legal issues. In cases of epidemics of the bees, also areas with prohibited access/trespassing can be defined, special facilities (e.g. “Belegstellen”) can be isolated, and even the colonies movements/translations of the beehives can be tracked and also administrated.

- GIS can also be used in typical bee related prevention scenarios, where only isolation, coordinated and punctual actions together with a full-coverage therapy will guarantee the success of the countermeasures. GIS can also work as a preventative tool for fighting future epidemics (e.g. Small Hive Beetle) not well

**METHODOLOGY FOR PREDICTING WINTER SURVIVAL OF HONEY BEE COLONIES**

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Apiculture is going through a period of change and new challenges on a worldwide scale. Parasitic mites, pest and disease resistance to control products, and the suspected increased effects of viruses are just a few of the factors that are negatively influencing honey bee health and survival. Coupled with land use conflicts and pollination demands, pest management for honey bees is becoming increasingly complex. Apiculture services and education of new beekeepers are not keeping pace with need and in fact are decreasing. Thus, our attempts to understand and manage bee health are failing to a certain degree. In recent years, many single factor studies have not been able to answer the questions that would solve the mystery of bee losses. Subsequent multifactor studies have been more enlightening and have determined that many factors are involved in what Rogers calls a Multiple and Variable Causative Agent Syndrome (MVCAS). However, the problem of measuring and interpreting the many factors that may be involved in losses is still a challenge. The following report outlines a methodology that has been used successfully for predicting winter survival of honey bee colonies in North America. Key components of the system are timing and thoroughness of assessment and diagnosis, comparison of results to provisional stratified thresholds, determining if factor interactions exist, and rating the survival chances of individual representative colonies. Using the method outlined, it now appears that bee health can be understood, losses can be predicted with relative accuracy, and knowing both of these points provides the knowledge required to take corrective action to prevent losses.
LENDING FOR MACEDONIAN BEEKEEPING DEVELOPMENT

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During the period between October, 2003 and April, 2006 (30 months), through the International Fund for Agricultural Development (IFAD) commercial credit line in the Republic of Macedonia, 313,792.74 EUR were disbursed to 73 beekeepers-producers and 3 legal entities (processing and trade of honey) as a direct investments.

The results from the statistical analysis of the data, shows that 72.6\% of the beekeepers-producers (borrowers) mainly are investing in purchasing new bee colonies, 21.9\% in new hives, 2.7\% in working capital, 1.3\% in honey packaging and 1.3\% in winter feeding.

Also, the paper presents analysis regarding regional distribution of the loans, amounts of the loans, gender balance of the borrowers, production level, financial impact on the production, employment generation and achieved development after loans disbursements etc. The data are analyzed within the beekeeping sector in comparison with other agricultural sectors which are covered by IFAD credit line in the Republic of Macedonia.

MEASURING OF CAPPED BROOD AREAS AT HONEY BEE COLONIES

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Colony population development is one of the most important criteria on scientific studies at honeybee colonies. A commonly used division is to estimate capped and uncapped brood areas. At this point of view, it is important to measure brood area in colony beside frame of bees. Brood area that contained eggs, larvae, and pupae on the combs in each bee colony has been measured as its size (cm\(^2\)) using the Photoshop programme on the computer. Assessing a whole colony for strength provides a very good snapshot in time of colony size, brood production and food status. Measurements of worker brood area are determined by measuring capped brood to the nearest cm\(^2\) using Adobe Photoshop\textsuperscript{®} CS2 9.0. This method based on estimating capped and uncapped brood. The estimation technique may take a bit of getting used to, but with experience it can be done quickly and accurately. The total square centimeter of brood for each side of the comb is estimated with this method.
PRODUCTION OF ROYAL JELLY IN QUEENRIGHT COLONIES WITHOUT RESTRICTION OF THE QUEENS

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Queenright colonies in a single super without any restriction of their queens were used for the production of royal jelly for a three-year period. The experiment started the first year with three colonies headed by one-year-old queens, two groups, during the second year, of three colonies each headed by one-year and two-year old queens respectively and three groups, during the third year, of three colonies each headed by one-year, two-year and three-year old queens.

Sixty queen cells were grafted every third day from June to October every year and the acceptance of the queen cells, the amount of royal jelly, the swarming impulse and the correlation of acceptance and queens’ age were recorded.

CURRENT STATUS OF BEEKEEPING IN THE EUROPEAN UNION

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On the area of EU more than 11 626 000 beehives are presented, from which 25% represent hives of 10 newly associated member states. Number of beekeepers is app. 630 000, from which 18 000 are considered as professionals. The highest bee density reaches Greece, Hungary and Slovenia. Annual honey yield is app. 160 000 tons, additional 170 000 tons are imported into EU from third countries. Average honey consumption per year and person is 0.66 kg. Main threats of European beekeeping are retiring of beekeeping sector, new bee diseases and declining of wholesale honey prices on inland market.

THE FEEDING OF HONEY BEE COLONIES WITH MALTOSE RICH STARCH SYRUP

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Investigations were carried out from June 2004 to April 2006 at 7 climatically different locations each with 12-21 colonies. The development of the colonies in the late summer and fall and their overwintering depended very strongly on the location.

Feeding of honey bee colonies with the starch syrup Meliose® in comparison with sugar water (3:2) did not affect the development of the colonies during and after the feeding period (in August/September) including wintering and the drawing out of foundation. The use of the syrup with a protein addition also remained without effect. This is valid both for the serving of the food in small and large portions in the late summer to increase the colony's stores for overwintering and for the feeding of nuclei and artificial swarms during their growth phase in summer.
The colonies fed with Meliose® processed without problems the syrup to winter food which did not show increased tendency towards the crystallization in comparison with the winter food produced from sugar water. Meliose® has two advantages: Colonies can be fed sufficiently with a single 20 liter portion. The syrup does not become ruined even if it is kept longer than 1 year.

**THE BEE-HOUSE OF J.G.MENDEL IN BRNO (CR) - THE HISTORICAL POINT OF THE EUROPEAN BEEKEEPING**

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The discoverer of genetical laws J.G.Mendel (1922-1884) was an active apiculturist, who also participated in the social life of beekeepers.

In the Augustinian Monastery in Brno he learned how to keep bees and later on, during the leadership of F. Zivansky, Mendel took part in guiding the Moravian Association of Beekeepers as the vice-chair and contributed to the high level of both, the theoretical and practical beekeeping of that time.

In 1971 he let to build a modern bee-house with the store, workroom and room for 15 bee hives and the cellar.

He kept bees in modern hives with frames, and enlarged their dimensions using only 2 instead of 4 original storeys. His colonies were wintered as well ventilated, strong in bees. Mendel investigated the nectar producing flora and possibilities of bees to use the nectar flow.

He tested all available honey bee races, and tried to get mating between young queens and selected drones in cages - probably with the aim to continue with his crossing trials also in bees.

Recently, the building has been restored, and equipped with various types of hives, it became an example of the beekeeping from Mendel's time till now. More details at: http://www.mendel-museum.org/; http://www.sci.muni.cz/ptacek/mendeluv-vcelin.htm

The presentation was possible due to the financial support from NAZV 44014.

**APICULTURAL VALUE OF SUNFLOWER HYBRIDS IN HUNGARY**

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Seed production of sunflower hybrids depends on honeybees as pollinators. Honeybees make foraging preferences among sunflower hybrids. In the years of 2002-2005 nectar production, nectar sugar concentration and foraging preference of honeybees among sunflower hybrids was studied in Hungary. During flowering period 7 hybrids were regularly sampled. According to our results weather conditions have high
importance in nectar production. The experimental data show the existence of hybrids with high nectar production and stabil apicultural value every year, irrespectively of the ecological conditions. In the experimental period the mean nectar amount ranged between 0.082-0.353 mg/flower/day. The sugar concentration varied between 36.8-58.7 % in the samples. Correlations were found between the amount and concentration of nectar as well as the temperature and relative humidity.

RESULTS OF THE TECHNOLOGY OF THE ROTATING BROOD-NEST HIVE (RBNH)

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In order to reduce chemical input functionality of the rotating brood-nest hive (RBNH) was investigated. The new invention – the rotation – keeps the mite development low. The technological improvement was achieved by automatisation of the rotation and with changed frame size. In 2001, we kept nine colonies in RBNH without any chemical treatment during the season. Mite population was diagnosed in late autumn. In the broodless period diagnostic treatment showed that in the experimental hives the lower number of the mites differing than the conventionally treated colonies. During June-August in 2002 we monitored the mite infestation in the brood and on the adult bees. In the next year the experiment was continued with 44 RBNH in Öttevény (Hungary). This productive apiary had average honey yield of 100 kg per RBNH (between 85-120 min-max values) in 2004 and in 2005. During the pre-winter inspection in autumn the bee population was on ten frames which can constitute a wintering-cluster on 6-7 frames. The overwintering loss caused by different factors did not reach 10%. After the blooming season varroa control can be done with regular registered acaricides. Thus RBNH have decreased mite infestation and colonies can start wintering with better chances.

As a conclusion RBNH had lower mite population. Rotation and handling of the brood and the honeycombs does not impair the behavior of the colony, making possible a clearer separation of nest and honey.
BEE SHOP session

6FP project PL 022568

Symposium organized by Robin Moritz

STRUCTURAL FEATURES OF SOME ROYAL JELLY PROTEINS AND PEPTIDES IN RELATIONSHIPS TO THEIR ANTIMICROBIAL ACTIVITY

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Proteins and peptides present in honeybee larval food play a significant role in honeybee life. They are not only source of nutrition, because of high content of essential amino acids for honeybee larvae, but they participate also in protection of honeybee larvae against pathogenic microorganisms. The physiological functions of royal jelly (RJ) proteins and peptides have not been exactly defined yet because a fundamental structural data are not available so far.

We have found that apalbumin 1, the most abundant protein of RJ, is present in different forms: as a monomer, an oligomer subunits and water-insoluble aggregates as result of its interaction with fatty acids. MALDI-TOF MS analysis confirmed that apalbumin 1 and RJ peptide apisimin form a stable heterodimer.

An example of biological activity in relationship to the structures of some RJ peptides can be demonstrated on the antibacterial activity of apisimin in comparison to royalisin. Basic peptide royalisin, as other members of insect defensin’s family has well-defined structure, stabilized by three disulphide bridges, while acidic serin-valin rich peptide apisimin creates predominantly helical structure with tendency to form oligomeric structures. Antibacterial activity of apisimin was observed only in its monomeric form (Bilikova et al, unpublished). Other example of biological potential of RJ proteins is stimulation of TNF-α release by monomeric apalbumin-1 in and apalbumin 2, but only low effect was observed in the case of oligomeric apalbumin-1 and/or apisimin, probably because amino acids sequential motifs of the apalbumin-1, responsible for stimulation of TNF-α release are blocked by protein-protein interactions.

Acknowledgment

This work was supported by VEGA grant Agency of Ministry of Education of Slovak Republic and Slovak Academy of Sciences No.: 2/4059/04, Max-Planck Society for Partner Group of Slovak Academy of Sciences and by grant of 6RP EU-BeeShop No.: 022568.
**HONEY BEE OR VARROA MITE ADAPTATIONS IN COEXISTING POPULATIONS?**

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An isolated and non-managed honey bee population on the island Gotland in the Baltic Sea has been kept for over 7 years without treatments for mite control. After heavy colony losses the second, third and fourth year of the experiment colony vitality is increasing with lower winter mortality, increased swarming rates and decreased mite infestation rates. A field experiment has been set up to investigate if the host-parasite co-adaptation that has occurred is mainly based on mite or bee related characteristics. Mite population dynamics, using two different sources of mites, is studied in honey bees that survive without mite control and in colonies where mite levels have been continuously controlled. The first results from these investigations will be reported.

**VIRUS TRANSMISSION IN THE PRESENCE AND ABSENCE OF VARROA DESTRUCTOR AND ITS EFFECTS ON BROOD AND ADULT BEES**

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Several hive-based experiments were conducted to investigate the effects of virus transmission by bees and by mites on a range of important colony parameters, such as the survival rates of open and closed brood; the capping and emergence rates and the longevity of adult bees. A quantitative nucleic acid-based assay was developed to monitor the titres of several important bee viruses simultaneously, to replace the ELISA assays used in earlier experiments. Hive experiments using naturally established infections were supplemented with laboratory and hive experiments of single virus infections aimed at quantifying the transmission efficacies of different viruses, both by oral and mite-mediated transmission. Data from these earlier experiments shows elevated levels of larval and pupal removal related to exposure to viruliferous bees during the larval stage, although the capping and emergence rates appear largely unaffected. Mite-mediated virus transmission was far more efficient, with a slight peak in pupal susceptibility during the middle of the pupal period. Updated data using greater replication and the improved virus assays will be presented.

**GENETIC VARIANCE IN HONEYBEE LARVAL RESISTANCE TO AMERICAN FOULBROOD**

*Dieter Behrens, Eva Forsgren, Ingemar Fries, Robin Moritz*

In-vitro reared drone larvae of several sister queens from an Apis mellifera ligustica and a Buckfast breeding line were infected with Paenibacillus larvae (type strain ATCC 9545) causing American Foulbrood (AFB). Although drone larvae were susceptible to AFB and could be reinfected under in-vitro conditions there were differences
within and between lineages. Infection resulted in a significant increase in mortality in the A. m. ligustica line but not in the Buckfast line. Different infection thresholds were found among sister queens of the A. m. ligustica line suggesting a considerable genetic variance for larval resistance against AFB. The results support a genetic model where a single gene is responsible for differences in susceptibility to infection.

**ON THE GENETICS MAP**

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The current honey bee linkage map comprises 2,008 microsatellite markers genotyped on 100 to 200 workers. The genetic length of the 16 linkage groups is 4,290, 4,114 and 3,902 cM respectively with the Haldane function of distance, the Kosambi function and the number of observed crossovers and hence its resolution is about 2 cM. Every genetic distance is below 10 cM. The map was used by the Baylor Center to organise 627 scaffolds of the sequence totalling 186 Mb and to orient most of them. A genetic map is the obligate complement of a sequence for association mapping studies but it may be associated to a haplotype map that depicts the pattern of linkage disequilibrium in natural populations. Haplotype maps are shaped by the variation of recombination along chromosomes, the regions of linkage equilibrium (steps) corresponding to hot spots and the regions of disequilibrium (blocks) to cold spots. We have taken advantage, for the two smallest chromosomes (15 and 16), of a map already more dense than that for the other chromosomes and of the superscaffolding effort (Robertson et al.). Only 5 and 4 gaps, probably very short, remain in the superscaffolded sequence of chromosomes 15 and 16 respectively. We increase the density of markers to reduce every distance to 1 or 2 cM and extend the number of genotyped individuals (300 for the moment and in a short future 500). This will lead to precisely map the hot and cold spots common to all progenies on these chromosomes. In parallel, drones from a natural population are genotyped to construct a haplotype map. Because they are haploid, they offer the great advantage to provide directly the picture of linkage (dis)equilibrium and avoid the problem of double heterozygote markers in diplotypes. The main interest of these haplotype map is to provide a guideline for the choice of markers used to localise mendelian genes and QTLs since it is not useful, for a whole genome scan, to saturate with numerous markers the regions that are in strong linkage disequilibrium. Joined to the possibility of using bulk segregant analysis with drones (or monoandric families), these maps will considerably reinforce the efficiency of further genetic analyses.
IS EUROPE A DESERT FOR HONEYBEES?

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The density of wild honeybee colonies (Apis mellifera) in the African deserts Sahara and Kalahari, as well as in two German national parks (Müritz and Hochharz), one city (Halle/Saale) and various apiaries, was estimated based on the genotypes of drones and worker offspring from mated queens. Estimated densities ranged between 4.5 - 6.4 colonies per square kilometer for the African deserts, being significantly higher than the colony densities in Germany, with estimates of 2.4 -3.2 colonies per square kilometer. These values closely match the nation wide density of colonies kept by beekeepers, showing that the densities of colonies observed in wild populations under the harsh conditions of the African deserts, exceed by far those observed in central Europe. We conclude that the apicultural activities in Europe are unlikely to compensate for the loss of habitats suitable for wild honeybees due to agriculture, forestry and other cultivation of land.

POPULATION DYNAMICS AND VARROA TOLERANCE FACTORS IN HONEY BEE COLONIES PRESELECTED FOR VARROA TOLERANCE

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The so called “Bond-Project” performed at an isolated part of the island of Gotland in the Baltic Sea has confirmed that European honey bee colonies can survive without Varroa treatment for over 7 years. We recorded the population dynamics of Varroa infested honey bee colonies headed by queens which were bred and mated at Gotland together with non-selected control colonies from Hohenheim at an isolated military camp in South Germany. The first experiment during the season 2004 (queens from 2003) already indicated a lower increase of Varroa population throughout the season and a better fitness of the “Gotland-colonies” (n=7) under high infestation pressure compared to control colonies (n=9). At the end of 2005 we started a repetition of this experiment with a total of 25 colonies. We included the comparative investigation of some tolerance factors like reproductive capacity of Varroa females and hygienic behavior of the bees.

First results will be presented including a critical evaluation of the methods used for host and parasite population dynamic measurements.
Supplements

PESTICIDE TRANSPORT WITH NECTAR AND POLLEN LOADS FOLLOWING TREATMENTS IN DIFFERENT BLOOMING CULTIVATIONS

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Moulds, bacteria and insects appearing also during blooming phases of rape, pome and stone fruits are treated with pesticide applications during the blooming phase. Only non toxic preparations are tolerated for this purpose. Such applications don’t have visible effects on the foraging behaviour of bees. The pesticides are collected together with nectar and pollen and are transported into the beehive. By means of tent tests with three different plants (Phacelia tanacetifolia, Borago officinalis, Sinapis alba), a field study with Brassica napus var. napus and laboratory studies, the individual transport of three pesticides (Vinclozoline, Boscalid alpha-Cypermethrin) in the honey sac and pollen loads of returning forager bees was determined.

After the application measurable residues in the collected nectar and pollen were found over a period of several days. High levels of the fungicides in the range of ppb up to ppm were found during the trials in the different cultures.

The amounts carried by single foragers in nectar and pollen showed strong variation for the total amounts and the proportion of the three substances. The pattern of contamination and degradation of pesticides varies in pollen and nectar of different plants. Knowing the amounts of pesticides transported into the beehive, the exposure of forager bees, hive bees and bee brood to the active ingredient can be calculated. These experiments make data available for further in vitro studies or feeding tests.
### AUTHORS INDEX

<table>
<thead>
<tr>
<th>Author</th>
<th>Pages</th>
<th>Author</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ackar D</td>
<td>111</td>
<td>Beneš K</td>
<td>19, 44, 48</td>
</tr>
<tr>
<td>Afik O</td>
<td>52, 91</td>
<td>Bieńkowska M</td>
<td>36, 57, 58</td>
</tr>
<tr>
<td>Angeli G</td>
<td>92</td>
<td>Biesmeijer K</td>
<td>5, 69</td>
</tr>
<tr>
<td>Alahiotis S</td>
<td>65</td>
<td>Bigler F</td>
<td>86</td>
</tr>
<tr>
<td>Almeida-Muradian LB</td>
<td>130</td>
<td>Bliková K</td>
<td>142</td>
</tr>
<tr>
<td>Alonso-Torre SR</td>
<td>125, 126</td>
<td>Billen J</td>
<td>55</td>
</tr>
<tr>
<td>Alvares R</td>
<td>46</td>
<td>Biris T</td>
<td>135</td>
</tr>
<tr>
<td>Alves MLTMF</td>
<td>130</td>
<td>Blacquière T</td>
<td>10</td>
</tr>
<tr>
<td>Arculeo P</td>
<td>39</td>
<td>Blanco-Contreras E</td>
<td>94</td>
</tr>
<tr>
<td>Arikawa K</td>
<td>9</td>
<td>Blanchard P</td>
<td>21, 23, 24</td>
</tr>
<tr>
<td>Arnold G</td>
<td>59, 87, 88</td>
<td>Blachtein B</td>
<td>69</td>
</tr>
<tr>
<td>Aronne G</td>
<td>119</td>
<td>Bodur C</td>
<td>63</td>
</tr>
<tr>
<td>Ashiralieva A</td>
<td>37</td>
<td>Boecking O</td>
<td>135</td>
</tr>
<tr>
<td>Aubert MFA</td>
<td>18, 22, 45, 80, 84, 88</td>
<td>Cordova-Serrano J</td>
<td>99</td>
</tr>
<tr>
<td>Aumeier P</td>
<td>46</td>
<td>Bommarco R</td>
<td>93</td>
</tr>
<tr>
<td>Aupinel P</td>
<td>82</td>
<td>Borris S</td>
<td>37</td>
</tr>
<tr>
<td>Aydin L</td>
<td>31</td>
<td>Bortolotti L</td>
<td>84</td>
</tr>
<tr>
<td>Azedo RAB</td>
<td>130</td>
<td>Bosch J</td>
<td>70, 78</td>
</tr>
<tr>
<td>Babendreier D</td>
<td>74, 86</td>
<td>Bouga M</td>
<td>61, 65</td>
</tr>
<tr>
<td>Baggio A</td>
<td>32, 66, 86</td>
<td>Božek M</td>
<td>100</td>
</tr>
<tr>
<td>Bach Kim Nguyen</td>
<td>89</td>
<td>Brasse D</td>
<td>79</td>
</tr>
<tr>
<td>Bąk B</td>
<td>57</td>
<td>Bratkovski J</td>
<td>57</td>
</tr>
<tr>
<td>Bakhshi AK</td>
<td>107, 120</td>
<td>Brindza J</td>
<td>99, 100</td>
</tr>
<tr>
<td>Ball B</td>
<td>22</td>
<td>Brodschneider R</td>
<td>14</td>
</tr>
<tr>
<td>Baranec T</td>
<td>99, 100</td>
<td>Bubalo D</td>
<td>123</td>
</tr>
<tr>
<td>Barouz D</td>
<td>69</td>
<td>Buckner J</td>
<td>78</td>
</tr>
<tr>
<td>Barros L</td>
<td>46, 115, 121</td>
<td>Büchler R</td>
<td>19, 44, 48</td>
</tr>
<tr>
<td>Bataintha A</td>
<td>77</td>
<td>Burdová M</td>
<td>117</td>
</tr>
<tr>
<td>Bázgerová E</td>
<td>41</td>
<td>Bzdil J</td>
<td>41</td>
</tr>
<tr>
<td>Beckh G</td>
<td>122</td>
<td>Cadikovska L</td>
<td>138</td>
</tr>
<tr>
<td>Beckmann K</td>
<td>122</td>
<td>Cahlková L</td>
<td>75</td>
</tr>
<tr>
<td>Bednář M</td>
<td>40</td>
<td>Çakmak I</td>
<td>31, 60, 98</td>
</tr>
<tr>
<td>Behrens D</td>
<td>143</td>
<td>Cano-Rios P</td>
<td>94, 96</td>
</tr>
<tr>
<td>Becher M</td>
<td>16</td>
<td>Caracappa S</td>
<td>62</td>
</tr>
<tr>
<td>Békési L</td>
<td>83, 141</td>
<td>Carré G</td>
<td>93</td>
</tr>
<tr>
<td>Benada O</td>
<td>27</td>
<td>Carrero P</td>
<td>112</td>
</tr>
<tr>
<td>Benard, J.</td>
<td>8</td>
<td>Cauich O</td>
<td>71</td>
</tr>
<tr>
<td>Benedetti S</td>
<td>107</td>
<td>Cavia MM</td>
<td>125, 126</td>
</tr>
<tr>
<td>Bermadou A</td>
<td>14</td>
<td>Celle O</td>
<td>21, 23, 24</td>
</tr>
<tr>
<td>Berthoud H</td>
<td>18, 29</td>
<td>Chaouch S</td>
<td>24</td>
</tr>
<tr>
<td>Bertoncelj J</td>
<td>129, 130</td>
<td>Charrière J-D</td>
<td>18, 29</td>
</tr>
<tr>
<td>Bessi E</td>
<td>31</td>
<td>Chauzat M-P</td>
<td>30, 45, 80, 84</td>
</tr>
<tr>
<td>Bianu E</td>
<td>85</td>
<td>Chioveanu G</td>
<td>38</td>
</tr>
<tr>
<td>Name</td>
<td>Page Numbers</td>
<td>Name</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------</td>
<td>-----------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Chittka L</td>
<td>7, 68</td>
<td>Drajnudel P</td>
<td>45, 88</td>
</tr>
<tr>
<td>Chlebo R</td>
<td>94, 99, 115, 139</td>
<td>Dražić M</td>
<td>113</td>
</tr>
<tr>
<td>Chmielewski W</td>
<td>103</td>
<td>Drezner - Levy T</td>
<td>11</td>
</tr>
<tr>
<td>Chouza M</td>
<td>114</td>
<td>Duangphakdee O</td>
<td>77</td>
</tr>
<tr>
<td>Chuda-Mickiewicz B</td>
<td>57</td>
<td>Duncan M</td>
<td>43</td>
</tr>
<tr>
<td>Cornelissen B</td>
<td>10</td>
<td>Ehrhardt K</td>
<td>19, 44, 48</td>
</tr>
<tr>
<td>Cornuet J-M</td>
<td>51</td>
<td>Eischen FA</td>
<td>94, 96</td>
</tr>
<tr>
<td>Correia D</td>
<td>116</td>
<td>Emmanouel NG</td>
<td>61</td>
</tr>
<tr>
<td>Costa C</td>
<td>34</td>
<td>Emsen B</td>
<td>138</td>
</tr>
<tr>
<td>Cralisheim K</td>
<td>12, 14</td>
<td>Estevinho L</td>
<td>115, 121</td>
</tr>
<tr>
<td>Crewe RM</td>
<td>42, 145</td>
<td>Faucon J-P</td>
<td>21, 23, 24, 30, 45, 80, 84, 88</td>
</tr>
<tr>
<td>Cuich O</td>
<td>71</td>
<td>Fenoy S</td>
<td>32</td>
</tr>
<tr>
<td>Cutler GC</td>
<td>80</td>
<td>Ferrazzi P</td>
<td>97</td>
</tr>
<tr>
<td>Czeckońska K</td>
<td>39</td>
<td>Fernández-Muiño MA</td>
<td>125, 126</td>
</tr>
<tr>
<td>Čačić F</td>
<td>107</td>
<td>Ferrero R</td>
<td>97</td>
</tr>
<tr>
<td>Čermák K</td>
<td>67</td>
<td>Flanjak I</td>
<td>123</td>
</tr>
<tr>
<td>Čermaková T</td>
<td>25, 111</td>
<td>Fonseca S</td>
<td>118</td>
</tr>
<tr>
<td>Daeseleire E</td>
<td>117, 118</td>
<td>Forsgen E</td>
<td>143</td>
</tr>
<tr>
<td>Dag A</td>
<td>52, 91</td>
<td>Forster R</td>
<td>79</td>
</tr>
<tr>
<td>Darkshifar I</td>
<td>25</td>
<td>Fortini D</td>
<td>82</td>
</tr>
<tr>
<td>Darvas B</td>
<td>83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>de Almeida Muradian LB</td>
<td>109, 130</td>
<td>Fournier D</td>
<td>13</td>
</tr>
<tr>
<td>de Jezús Hernández M</td>
<td>76</td>
<td>Fournier D</td>
<td>13</td>
</tr>
<tr>
<td>de la Rúa P</td>
<td>59</td>
<td>Fries I</td>
<td>22, 143, 145</td>
</tr>
<tr>
<td>de Lorenzo Carretero C</td>
<td>103, 124</td>
<td>Fuchs S</td>
<td>28, 60, 66, 77</td>
</tr>
<tr>
<td>de Miranda J</td>
<td>143</td>
<td>Fujiyuki T</td>
<td>20</td>
</tr>
<tr>
<td>de Pinho L</td>
<td>14</td>
<td>Gallina A</td>
<td>66, 86</td>
</tr>
<tr>
<td>Decourtye A</td>
<td>64, 81, 83</td>
<td>García-Palencia P</td>
<td>32, 34, 35, 36</td>
</tr>
<tr>
<td>Delagulla C</td>
<td>32</td>
<td>Garido ME</td>
<td>34, 35</td>
</tr>
<tr>
<td>Delphine DN</td>
<td>125</td>
<td>Garnery L</td>
<td>59, 88</td>
</tr>
<tr>
<td>Denisov B</td>
<td>101</td>
<td>Garreau L</td>
<td>13</td>
</tr>
<tr>
<td>Deowanish S</td>
<td>77</td>
<td>Garrido C</td>
<td>19, 44, 48</td>
</tr>
<tr>
<td>Derakhshifar I</td>
<td>25</td>
<td>Garrido-Bailón E</td>
<td>33</td>
</tr>
<tr>
<td>Dewenter IS</td>
<td>93</td>
<td>Gauthier L</td>
<td>21</td>
</tr>
<tr>
<td>Di Bernardo ML</td>
<td>112</td>
<td>Gauthier M</td>
<td>13, 14</td>
</tr>
<tr>
<td>Di Noto AM</td>
<td>39</td>
<td>Gencay Ö</td>
<td>110</td>
</tr>
<tr>
<td>Dias LG</td>
<td>115, 116, 121</td>
<td>Gençer HV</td>
<td>53, 56</td>
</tr>
<tr>
<td>Dietemann V</td>
<td>11, 42, 145</td>
<td>Genersch E</td>
<td>6, 24, 37</td>
</tr>
<tr>
<td>Dimitrov L</td>
<td>138</td>
<td>Gerritsen L</td>
<td>49</td>
</tr>
<tr>
<td>Dimou M</td>
<td>98</td>
<td>Gerula D</td>
<td>36, 57, 58</td>
</tr>
<tr>
<td>Doberšték U</td>
<td>129, 130</td>
<td>Giacomello F</td>
<td>92</td>
</tr>
<tr>
<td>Dobrynin N</td>
<td>63</td>
<td>Girante S</td>
<td>115, 121</td>
</tr>
<tr>
<td>Dogaroglú M</td>
<td>129</td>
<td>Giurfa M</td>
<td>8, 13</td>
</tr>
<tr>
<td>Dollin A</td>
<td>43</td>
<td>Goksoy AT</td>
<td>98</td>
</tr>
<tr>
<td>Domagaj Matković</td>
<td>121</td>
<td>Goleb T</td>
<td>129, 130</td>
</tr>
<tr>
<td>Donders J</td>
<td>10, 28</td>
<td>González I</td>
<td>112</td>
</tr>
<tr>
<td>Dovč P</td>
<td>74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Authors index</td>
<td>Pages</td>
<td>Authors index</td>
<td>Pages</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------</td>
<td>--------</td>
<td>-------------------------------------------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>González Lorente M</td>
<td>103, 124</td>
<td>Janke M</td>
<td>135</td>
</tr>
<tr>
<td>Goras G</td>
<td>139</td>
<td>Janoušová P</td>
<td>40</td>
</tr>
<tr>
<td>Gorgulu O</td>
<td>128</td>
<td>Jasinski Z</td>
<td>57</td>
</tr>
<tr>
<td>Gosterit A</td>
<td>73</td>
<td>Jojczyk A</td>
<td>57</td>
</tr>
<tr>
<td>Greco M</td>
<td>43</td>
<td>Joller D</td>
<td>86</td>
</tr>
<tr>
<td>Gregorc A</td>
<td>49</td>
<td>Jones R</td>
<td>132</td>
</tr>
<tr>
<td>Grzechnik K</td>
<td>119</td>
<td>Jordanova L</td>
<td>15</td>
</tr>
<tr>
<td>Gspurning J</td>
<td>136</td>
<td>Kahkonen B</td>
<td>143</td>
</tr>
<tr>
<td>Gul A</td>
<td>120</td>
<td>Kahya Y</td>
<td>56</td>
</tr>
<tr>
<td>Gulduren Z</td>
<td>54</td>
<td>Kalinová B</td>
<td>75</td>
</tr>
<tr>
<td>Gurel F</td>
<td>73</td>
<td>Kamler F</td>
<td>134</td>
</tr>
<tr>
<td>Gutiérrez L</td>
<td>112</td>
<td>Kantiková M</td>
<td>25, 111</td>
</tr>
<tr>
<td>Habovštiaková J</td>
<td>25</td>
<td>Kapustjanskij A</td>
<td>72</td>
</tr>
<tr>
<td>Haddad N</td>
<td>66, 77</td>
<td>Karasu A</td>
<td>98</td>
</tr>
<tr>
<td>Hak J</td>
<td>135</td>
<td>Karpidou V</td>
<td>92</td>
</tr>
<tr>
<td>Haklová M</td>
<td>40, 41</td>
<td>Kaspar F</td>
<td>67</td>
</tr>
<tr>
<td>Halm MP</td>
<td>87</td>
<td>Kastberger G</td>
<td>75</td>
</tr>
<tr>
<td>Hamm A</td>
<td>89</td>
<td>Kefuss J</td>
<td>132</td>
</tr>
<tr>
<td>Haristoros L</td>
<td>92</td>
<td>Kekecoglu M</td>
<td>61</td>
</tr>
<tr>
<td>Harizanis P</td>
<td>61</td>
<td>Kemp WP</td>
<td>70, 78</td>
</tr>
<tr>
<td>HärTEL S</td>
<td>42</td>
<td>Kence A</td>
<td>54, 63</td>
</tr>
<tr>
<td>Hatjina F</td>
<td>61, 92</td>
<td>Kence M</td>
<td>54, 63, 65</td>
</tr>
<tr>
<td>Haueter M</td>
<td>18</td>
<td>Kenjerić D</td>
<td>107, 123</td>
</tr>
<tr>
<td>Hernández M</td>
<td>76</td>
<td>Kezić N</td>
<td>113</td>
</tr>
<tr>
<td>Hernández-Garcia R</td>
<td>59</td>
<td>Kiliias G</td>
<td>65</td>
</tr>
<tr>
<td>Higes M</td>
<td>30, 32, 33, 34, 35, 36</td>
<td>Kinoshita M</td>
<td>9</td>
</tr>
<tr>
<td>Hirschmugl M</td>
<td>75</td>
<td>Kiprijanovska H</td>
<td>138</td>
</tr>
<tr>
<td>Hofbauer J</td>
<td>48</td>
<td>Kirchner WH</td>
<td>46</td>
</tr>
<tr>
<td>Hoffmann D</td>
<td>43</td>
<td>Klee J</td>
<td>27</td>
</tr>
<tr>
<td>Hori S</td>
<td>9</td>
<td>Klossa-Kilia E</td>
<td>65</td>
</tr>
<tr>
<td>Horn H</td>
<td>95</td>
<td>Koeniger G</td>
<td>1, 55</td>
</tr>
<tr>
<td>Hovorka O</td>
<td>75</td>
<td>Koeniger N</td>
<td>1, 77</td>
</tr>
<tr>
<td>Hrabák J</td>
<td>27, 40</td>
<td>Kögelberger H</td>
<td>25</td>
</tr>
<tr>
<td>Hrušková-Heidingsfeldová O</td>
<td>27, 40</td>
<td>Kölling-Speer I</td>
<td>122</td>
</tr>
<tr>
<td>Huettlinger E</td>
<td>75</td>
<td>Kolodziejek J</td>
<td>25</td>
</tr>
<tr>
<td>Huidobro J</td>
<td>125, 126</td>
<td>Koltowski Z</td>
<td>93</td>
</tr>
<tr>
<td>Human H</td>
<td>11, 102</td>
<td>König M</td>
<td>19</td>
</tr>
<tr>
<td>Ichikawa N</td>
<td>9</td>
<td>Konrad R</td>
<td>74</td>
</tr>
<tr>
<td>Imdorf A</td>
<td>18, 29</td>
<td>Kott T</td>
<td>67</td>
</tr>
<tr>
<td>Ings TI</td>
<td>68</td>
<td>Kovac H</td>
<td>14</td>
</tr>
<tr>
<td>Iscache A-L</td>
<td>21</td>
<td>Kozmus P</td>
<td>74</td>
</tr>
<tr>
<td>Ivanova E</td>
<td>12</td>
<td>Krakar D</td>
<td>113</td>
</tr>
<tr>
<td>Izquierdo F</td>
<td>32</td>
<td>Kralj J</td>
<td>28</td>
</tr>
<tr>
<td>Jabbarfarhoud H</td>
<td>65</td>
<td>Kraus FB</td>
<td>76, 145</td>
</tr>
<tr>
<td>Jaffe R</td>
<td>145</td>
<td>Krewenka KM</td>
<td>93</td>
</tr>
<tr>
<td>Jamnik M</td>
<td>129, 130</td>
<td>Krieg P</td>
<td>48</td>
</tr>
<tr>
<td>Jamnik M</td>
<td>129, 130</td>
<td>Kritikopoulou K</td>
<td>92</td>
</tr>
</tbody>
</table>
Kubersky U 135  Méndez J 114
Kubo T 9, 20  Mendil D 128
Kuhlmann M 70  Merkel K 43
Kuhn R 29  Meydan H 53
Kunová O 99, 100  Michaud B 82
Lallemand P 21, 23, 24, 25  Miklošiková Z 100
Lattorf HMG 53  Milani N 22
Lauber É 83  Mladenović M 55
Lazaridou E 139  Monnerot M 51
Leadbetter, E 7  Moors L 55
Lehrbach H 142  Moreno G 125, 126
Leonardi F 34  Morison N 93
Liebig G 46, 133, 139  Moretti ACC 130
Lichtenberg-Kraag B 106  Moritz RFA 15, 16, 22, 42
Lodesani M 34  44, 53, 143, 145
Lorenzo Lozano P 124  Morya M 130
Loublier Y 88  Mougel F 51, 144
Lüllmann C 122  Muñoz-Soto R 94
Lutzová M 40  Mustafa S 47
Lyapunov Y 114  Mutaers M 44
Maccagnani B 71, 92  Mutinelli F 32, 66, 86
Macias JO 71  Mutsaers M 135
Madras-Majewska B 57  Nanda V 107, 120
Magacz Z 99  Nava-Camberos U 96
Maini S 78  Ne’eman G 93
Maistrello L 34  Nedic Tiban N 111
Mamouzi A 92  Nedić N 55
Mandić ML 123  Nentchev P 15, 60
Mannino S 107  Neumann P 42, 43
Maori E 26  Nguyen BK 89
Marani G 34  Nica D 85
Marionneau R 14  Nicolson SW 11, 102
Martel A-C 80, 84, 88  Nomoto A 20
Martimianakis S 65  Nowotny N 25
Martin R 30, 32  Nőžkova J 99, 100
Martínek K 40  Odoux JF 82
Martin-Hernandez R 33, 34, 35, 36  Ohka S 20
Marty C 13  Ohmenhauser M 95
Masierowska M 131  Oliveira S 13
Mates V 135  Oliveira KCLS 130
Mato I 125, 126  Oliveri E 62
Meana A 30, 33, 34, 35, 36  Olivier V 21, 23, 24
Medrzycki P 84  Ono M 20
Meglič V 74  Ozzi JL 5
Meixner M 60, 66  Oz M 98
Melendez RV 71  Özdi F 53
Özkök Tüylü A 109  
Ozmen N 98  
Page R 4  
Palencia PG 32  
Panasiuk B 36, 57  
Paulus HF 72  
Paxton RJ 27, 92  
Pérez Martín RA 103, 124  
Perl A 107  
Perl-Pirički A 123  
Petkovski V 138  
Pettis JS 43  
Pflugfelder J 42  
Piętka T 131  
Pietzsch K 95  
Pilizota V 111  
Pistorius J 80, 146  
Pitts-Singer T 78  
Pohorecka K 36, 108  
Porrini C 84  
Portelli, G. 8  
Potts SG 93  
Prabucki J 57  
Primorac Lj 107, 123  
Ptáček V 72, 75, 140  
Quezada-Euan JJG 71  
Radioff SE 43  
Raine N 7, 68  
Rašić S 55  
Raymond-Delpech V 13  
Reale S 62  
Reinsch N 48  
Reybroeck W 117, 118  
Reyes-Carrillo JL 94, 96  
Ribière M 21, 23, 24  
Riessberger U 14  
Ritter W 30, 95  
Roberts SPM 93  
Rodríguez P 125, 126  
Rodríguez Martínez R 96  
Rodríguez-Malaver AJ 112  
Rodríguez-Raji FJ 114  
Roetschi A 29  
Rogers R 137  
Romaniuk K 50, 90  
Romeis J 86  
Rondón C 112  
Rortais A 59, 87, 88  
Rosenkranz P 47, 133, 143, 145  
Roša J 113  
Rybak-Chmielewska H 108  
Saavedra AR 112  
Saavedra O 112  
Sabattini AG 84  
Sahinler N 120, 128, 129  
Sahinler S 128  
Salih B 109, 110  
Samborski J 57  
Sandoz JC 14  
Sánchez D 76  
Sánchez MP 125, 126  
Sánchez-Bernal F 94  
Sancho MT 125, 126  
Sasaki N 9  
Scala M 119  
Scindler M 69  
Scott-Dupree CD 80  
Seijo M 114  
Sela I 26  
Semkiw P 96, 123  
Sener A 109  
Serrano J 59  
Sgolastra F 70, 78, 84  
Shafir S 11, 52, 91  
Shaibi T 145  
Schäfer M 38  
Scharpenberg H 15  
Schiendler M 69  
Schmickl T 12  
Schnell H 145  
Schroeder A 95  
Schurr F 21, 23, 24  
Siede R 19  
Silici S 128  
Sinacori A 62  
Siuda M 57  
Skorupski P 68  
Skowronek W 96, 123  
Skubida P 96, 123  
Slanická M 111  
Smith BH 11  
Sokol R 50  
Soland G 52, 62
<table>
<thead>
<tr>
<th>Name</th>
<th>Pages</th>
<th>Name</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solignac M</td>
<td>51, 144</td>
<td>Topolska G</td>
<td>22</td>
</tr>
<tr>
<td>Sorkun K</td>
<td>109, 110</td>
<td>Trautvetter S</td>
<td>122</td>
</tr>
<tr>
<td>Soylak M</td>
<td>128</td>
<td>Trostle GE</td>
<td>70</td>
</tr>
<tr>
<td>Soysal M</td>
<td>61</td>
<td>Tsirakoglou</td>
<td>92</td>
</tr>
<tr>
<td>Spaethe J</td>
<td>7, 72</td>
<td>Tunca RI</td>
<td>65</td>
</tr>
<tr>
<td>Speer K</td>
<td>122</td>
<td>Tuzen M</td>
<td>128</td>
</tr>
<tr>
<td>Spiewok S</td>
<td>43</td>
<td>Uzunov A</td>
<td>138</td>
</tr>
<tr>
<td>Spodniewska A</td>
<td>90</td>
<td>Vaissiere BE</td>
<td>93</td>
</tr>
<tr>
<td>Spooner-Hart R</td>
<td>43</td>
<td>Valdovinos-Nuñez GR</td>
<td>71</td>
</tr>
<tr>
<td>Stanislavjević L</td>
<td>55</td>
<td>Valterová I</td>
<td>75</td>
</tr>
<tr>
<td>Stawiarz E</td>
<td>119</td>
<td>van der Steen J</td>
<td>28</td>
</tr>
<tr>
<td>Staykova T</td>
<td>12</td>
<td>Vandame R</td>
<td>76</td>
</tr>
<tr>
<td>Steehmüller J</td>
<td>95</td>
<td>Vautrin D</td>
<td>51, 144</td>
</tr>
<tr>
<td>Steffan-Dewenter I</td>
<td>68</td>
<td>Vela Hortigüela L</td>
<td>103</td>
</tr>
<tr>
<td>Steidle H</td>
<td>47</td>
<td>Vesely V</td>
<td>134</td>
</tr>
<tr>
<td>Stern RA</td>
<td>52</td>
<td>Vierikova M</td>
<td>111</td>
</tr>
<tr>
<td>Steinerz M</td>
<td>72</td>
<td>Vilas Boas M</td>
<td>46, 115, 116, 118, 121</td>
</tr>
<tr>
<td>Strzalkowska M</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suárez-Lugue S</td>
<td>125, 126</td>
<td>Virant-Doberlet M</td>
<td>74</td>
</tr>
<tr>
<td>Subaric D</td>
<td>111</td>
<td>Vit P</td>
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<td>80, 146</td>
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