**ΠΑΡΑΣΚΕΥΗ: 29 Νοεμβρίου**

**POSTERS**

## POLLEN GRAIN ATLAS FROM THE HONEY PLANTS OF RHODES

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On the island of Rhodes between September 2001 and August 2002, 110 honey plants were collected, identified and classified according to their flowering time. Slides of their pollen grains were prepared according to Louveaux method (1970), photos from light microscope (HM) were taken through Olympus DP-soft 3.0 software. These were catalogued in a reference book.

This collection of pictures of pollen grains from the honey plants of Rhodes will help to determine the botanical and geographical origin of honey in Rhodes.

## POLLEN ATLAS OF PLANTS FROM CRETE (GREECE)

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During the last two years, reference pollen slides were prepared from more than 70 different taxa and 20 families of plants occurring in Crete. Digital pictures of pollen grains examined under optical microscope (with simple objectives or using Nomarski prisms, phase contrast and the fluorescent system) are presented, as well as pictures of the utilized plants that have been identified. Voucher specimens of these are preserved in the *Herbarium* of MAICh.

## IDENTIFICATION OF SUGARS IN HONEYDEW SECRETIONS BY *Marchalina hellenica*

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The research project “Factors that influence honeydew secretions on pine trees” (Ministry of Agriculture - E.C.1221/97) involves the determination of the sugar content of honeydew secretions by *Marchalina hellenica* (Gennadius) parasitising on most species of pine trees in Greece. The samples were collected in two periods: August - November and February - April. The frequency of sample collection depended on the stage in the biological cycle of the insect and the weather conditions. Honeydew secretions (drops) were collected directly from two species of pine trees (*Pinus halepensis* and *P.brutia*) with a micropipette. We have analysed the sugar content of these secretions by liquid chromatography (HPLC) for the first time.

A very high proportion of sucrose was found (up to 44.2%). Appreciable amounts of fructose and melezitose were present (up to 24.8 and 17.5 % respectively), and glucose and maltose were found in smaller amounts. These preliminary data have indicated significant differences between the samples collected at the same time and from the same area on one

species of pine tree. The intensity of honeydew secretions by *Marchalina hellenica* in both periods of collection varied greatly. Further investigation of these results could also provide information for predicting honeydew secretions on pine trees.

## MORPHOLOGICAL CHARACTERISTICS OF THYMUS POLLEN GRAINS FROM DIFFERENT AREAS OF GREECE

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In order to discover whether the geographical origin of thyme honey can be determined by reference to the morphological characteristics of the pollen grains that the honey contains, thyme plants of the islands of Crete, Rhodes, Kithira, Sikinos, Sifnos, Thasos and Cyprus were collected during 2000-2002. Slides of the pollen grains were prepared according to Louveaux method (1970), and photos from a light microscope (HM) were taken. The length, width, circumference and area of twenty pollen grains of each slide were measured through Olympus DP-soft 3.0 software.

The results of the statistical analysis indicated that it is possible to trace the geographical origin of thyme honey by examining the morphological characteristics of thyme pollen grains it contains.

## LIQUEFYING CRYSTALLIZED HONEY WITH ULTRASONIC WAVES

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Samples of crystallized honey were divided into 3 equal parts of one kg each. The first was liquefied by ultrasonic waves at 25 KHz, the second by heating at 60$^{o}$ C, until it became liquid and the third remained untreated. This study involved 15 samples of erica honey, 18 of cotton, 17 of thyme, 15 of blossom, 20 of sunflower, 16 of citrus, 14 of chestnut, 15 of pine honey blended with 30% erica honey and 14 fir honey blended with 30% erica honey.

All samples were analyzed for HMF and diastase activity. Results indicated that the quality of honey was better preserved when liquefied by 25 KHz ultrasonic treatment than by heating at 60$^{o}$ C.

## HONEY VOLATILE COMPOUNDS ISOLATION WITH PURGE & TRAP. THE VOLATILE FEATURES OF MONOFLORAL CATEGORIES OF GREEK HONEY

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Nectar and honeydew, from which honey is derived, contain characteristic volatile compounds. These volatile compounds are the main source of the aroma and could be a useful tool for determining the botanical origin of honey. These components are transferred to honey where they can be detected. Chemically, these substances are structurally complex (aldehydes,

acids, esters) and are identified by gas chromatography. The usual method for isolating them is steam distillation/solvent extraction (Likens-Nickerson method).

Recently some modern extraction techniques have been developed. In present work the volatile and semi volatile components have been isolated with the modern technique "Purge & Trap". After the extraction, gas chromatograph-mass spectrograph system is used for separation, detection and identification. The chromatograms show many volatile compounds, some of them characteristic for each type of honey.

## RHODES HONEY- PROSPECTS FOR THE ACQUISITION OF THE "GEOGRAPHIC ORIGIN LABEL"

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Ninety eight samples of honey were collected during three different periods (summer 2001, autumn 2001, summer 2002) from Rhodes island. The samples were analyzed for their physiochemical, microscopical and volatile characteristics. The Antioxidant and bacteriostatic activity of the honey was examined as well. The physiochemical characteristics (Diastase, HMF, electrical conductivity, moisture), indicate no difference between the honey of Rhodes and those of other regions.

The microscopical analysis, the antioxidant and bacteriostatic activity and the volatile components that characterize Rhodes honeys can be attributed to their different geographic origin. A hundred and six different volatile components were identified with a Gas Chromatography-Mass Spectrometry system, while eighteen of them were present in every honey that had been analysed.

These results mean that there are more prospects of it becoming possible to label honeys according to their geographical origins.

## HONEYDEW-PRODUCING INSECTS OF PINE TREES

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In order to examine the honeydew producing insects found in pine trees pine, tree cuttings were collected from Kassandra Halkidiki and from Thessaloniki twice every month in 2001. During these investigations, it was found that insects (coccids) other than *Marchalina hellenica*, the most important honeydew insect, also parasitise pines.

The insects were collected, photographed, preserved and given for identification. The identification will be done by specialists working in the a) Museum National d' Histoire Naturale –Paris, France and b) Dept. Of Entomology -the Volcano center -Bet Dagan, Israel. It is very important to have a number of the insects at all stages for identification. At least two of the insects found on pine trees were observed to secrete honeydew but no quantitative or

chemical analysis has been done yet on this honeydew. The biology, the ecology and the economic importance of these insects needs to be further investigated and determined.

## SIDE EFFECTS OF ACARICIDES USED AGAINST VARROA MITE ON HONEY BEE BEHAVIOR: HONEY PRODUCTION AND FOOD CONSUMPTION

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The effect of fluvalinate, coumaphos and malathion on the honey production, (in 36 hives) sugar cake consumption, (in 32 hives) and winter food consumption (in 36 hives) of *Apis melifera macedonica* was evaluated in three experiments using a total of 104 colonies. No difference was found between any of the experimental colonies and the controls after the application of all three acaricides.

The effect of fluvalinate and coumaphos on the amount of sucrose syrup consumed by honey bees kept in laboratory cages (27 cages, 50 bees per cage) was also tested. The average consumption of syrup in control cages was 28.73ml per bee every 6 hours. Where coumaphos was applied there was a significant reduction in the consumption of syrup (20.3 ml/bee/6hrs.), while where fluvalinate was applied the average consumption was reduced (25.67ml/bee/6hrs) but not significantly so.

## FLUCTUATIONS OF THE NUMBER OF LIGHTLY AND DARKLY PIGMENTED *Varroa destructor* (ACARI: VARROIDAE) IN THE HIVE DEBRIS OF COLONIES OF *Apis mellifera macedonica* (HYMENOPTERA: APIDAE)

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We have studied the fluctuation in the number of the lightly and darkly pigmented *Varroa destructor* in the hive debris of colonies of the Macedonian bee race *Apis mellifera macedonica* (Ruttner), for a period of two years, i.e. from April 1999 to April 2001. The results refer to 12 colonies placed in Langstroth hives with a removable bottom board equipped with a "trap" – tray for mites. The number of darkly or lightly pigmented mites fallen on hive debris were collected and were counted every 2 to 5 days. For each category, the total number of mites was calculated on an interval of 21 days.

The average number of lightly pigmented mites has shown an increase during the period from May to mid-October of the first year and a consecutive decrease towards late-April of the following year. In the second year, there was an increase from April to mid-August and a decrease up to late February of the following year. We have observed an increase in the average number of lightly pigmented mites during the remaining two periods of calculation. The average number of darkly pigmented mites has shown an increase from June up to the ten last days of November and a consecutive decrease up to late-April of the second year. Afterwards, there was an increase up to end-July and a decrease followed by some fluctuations up to mid-March. We have observed a light increase in the average number of darkly pigmented mites in the last period of calculation.

The fluctuations in the number of lightly and darkly pigmented mites collected from the hive debris are discussed in respect of colony-related parameters and the temperature of the

environment. Moreover, one will also find some conclusions regarding the effect of the mite's mortality rate on the development of its population.

## REINFESTATION OF HONEY BEES WITH VAROA MITES CAUSED BY DRIFITNG AND ROBBING

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10 hives which had been set out so as to reduce drifting were used to determine how drifting or robbing influences hive reinfestation by Varroa mites. The colonies were divided in two apiaries (A and B). The hives in apiary A were treated with Apistan strips for Varroa control. Apiary B, which was 100 m away from A was untreated. 400 color marked bees were put into each hive in both apiaries. Virtually no bees drifted. All those that did drift were possibly robbers, and all came from treated hives.

Untreated hives seemed to act as reservoirs of infection for the treated colonies. The significance of reinfestation, and the measures that need to be taken, (e.g large scale treatment for Varroa) especially when the hives are moved to pinewoods, where no measures for controlling drifting are taken, are discussed.

## SOME PHYSICOCHEMICAL CHARACTERISTICS OF FIR HONEYP RODUCED WITH THE MEDIATION OF THE INSECT *Marchalina hellenica* (Genadius)

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Honeydew honey samples from fir-trees from mountain Helmos (Greece, longitudine 22°11'33"E, latitudine 37°59'30"N, altitudine 2,200m), which were produced with the mediation of the insect *Marcalina hellenica* (Genadius) (Homoptera, Coccoidea, Margarodidae) were analyzed and examined for their physicochemical characteristics. The physicochemical characteristics were moisture content %, acidity, sacharides (sugars) (method Lane Eynon and Gas chromatography), hydroxy-methyl-furforal (HMF) and enzymic activity (diastase).

The results indicated that the values of moisture content %, acidity, diastase and HMF were inside the E.U. regulations. Reduced sugars determined by the Lane Eynon method were found to be slightly below E.U. regulations.

## TECHNICAL EDUCATION. THE CURRENT SITUATION IN GREECE

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During the last 20 years, the character and even the name of the vocational school in Greece has changed many times. These changes led finally to the Technical and Vocational Schools and the School Laboratory Centers that support these schools. Recently, a systematic effort was

made by the Ministry of Education to look into various essential issues, including, among other things, studies, infrastructure and professional rights. The decline in importance of the agriculture sector in the school year 2002-2003 is obvious, although this sector was one of the first sectors that was established in the technical schools in Greece.Starting from the concern of the educators in the sector of Agriculture about the future of the sector, we recorded data about the Technical School, in an effort to investigate the results of the laboratory education (comprehension, familiarization, impressions).

This research was conducted between 1993 and 2000. One hundred students in the last grade of the technical schools of Agia and Elassona in Central Greece were given a questionnaire with seven groups of questions. The questionnaire was designed to record the students' opinions about the education program, the school buildings, the facilities, the materials, the methods and the educators.The findings are revealing. The students chose the creative, suggested the innovative, preferred the different and disapproved of any discrimination in areas or schools. Students continually referred to the bee plants, the bee and the honeycomb products they learned about as part of the courses on environmental, health and consumer education.

We therefore propose that in the future technical schools should be experience based, in with students being in contact with people and equipment relevant to what they are studying outside in the real world where possible. We also suggest the better use of the educators from the agricultural sector inside and outside the school.

## FACTORS AFFECTING THE PRODUCTION OF ROYAL JELLY

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Royal jelly production was evaluated for two consecutive years, under the following conditions: a) queenless colonies without introduced eggs or larvae, b) queenless colonies with a comb or sealed brood added every 6 days, c) normal colonies where the queen was removed temporarily, d) triple- horizontal colonies with two queens. Additionally, royal jelly production was evaluated in artificial queen cells grafted with drone larvae of various ages.

Queenless colonies, which are not provided with sealed brood are not able to produce royal jelly for longer than 24 hours (8 graftings). In all the other experimental conditions, with or without a queen, royal jelly production continues for at least 6 months (56 graftings). Bees accept the cells with drone brood very well and produce a similar quantity and quality of royal jelly as those with worker brood. The age of the larva plays a very important role in the quantity of royal jelly produced.

## IMPORTANT CHARACTERISTICS OF THE MOST SUITABLE TYPE OF HIVE FOR GREECE

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Three types of hives were studied, 10 hives of each type, with a movable bottom board and Australian type of lid, were used. The first type of hive was the Langstroth hive, containing 10 standard (Hoffman) frames in the brood chamber and 10 frames in the honey super. In the second hive type the brood chamber and the honey super contained 8 standard frames each. The third type contained 9 frames which were a little shorter and deeper. All hive types were equal in terms of population brood, food and with sister queens. Hive management was the same in all three types.

After examining the three types of hive from the management point of view, we found the 10 and 8 frame hives are more convenient than those with 9 frames. This is because frames with standard 10 and 8 frame dimensions are readily available in Greece, and also other equipment designed to be used with these standard dimensions (eg honey extractor etc) can easily be obtained.

The 8 frame hive is preferable to 10 frame hive because is lighter and less strength is needed to handle it during inspection or moving to another location. But it was often necessary to add a third chamber. This additional chamber meant it was harder work to manage the hive. However, under Greek conditions, the10 frame hive does not require a third chamber. The 9 frame hive is less practical than the 8 and 10 frame hives. The importance of a young queen of good quality in every colony should be noted.

## INFLUENCE OF TEMPERATURE ON THE SEALED WORKER BEE BROOD OF *Apis mellifera L.*

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Cells of sealed worker brood of the honey bee *Apis mellifera macedonica* of ages 0 h, 50 h, 98 h and 193 h were inserted in an incubator after capping of the cells and left there until the end of the experiments. They were kept at different temperatures - 25$^{o}$C, 30$^{o}$C, 35$^{o}$C and 38$^{o}$C. It was found that both abnormal temperature conditions had a negative effect both on the length of time needed for brood development and on the percentage of emerging bees. Negative effects varied with the age of developing bee in the sealed cell. The non-normal temperature resulted in deformed wings too. In each phase of development, the further the temperatures varied from the normal temperatures, the worse the effect on the development of the sealed brood. However, the cells least affected were those at 193 hr and the most affected were those of the newly sealed brood. These results are discussed in connection with the use of a bee brood incubator in the beekeeping practice.

## A COMPARATIVE STUDY OF THE EXTERNAL MORPHOLOGY OF THE PROTHORACIC AND THE PROPODEAL SPIRACLES IN FOUR STRAINS OF *Apis mellifera* (L.). POSSIBLE RESISTANCE MECHANISM AGAINST THE TRACHEAL MITE *Acarapis woodi* (R.)

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The prothoracic and the propodeal spiracles of four strains of honey bees were examined with a light microscope. The propodeal spiracles were examined, using a scanning electron microscope as well. Certain dimensions, such as the length and the area of atrium in the prothoracic spiracle, were found to be significantly larger in *Apis mellifera ligustica*, *Apis mellifera carnica* and *Apis mellifera mellifera* compared to *Apis mellifera macedonica*. The density of hairs measured in a specific area, next to the prothoracic spiracle, was significantly higher in *Apis mellifera macedonica* and *Apis mellifera ligustica* compared to *Apis mellifera carnica* and *Apis mellifera mellifera.* The cross-diameter of the trachea was also significantly larger in *Apis mellifera ligustica* compared to *Apis mellifera macedonica.*

The dimensions of the propodeal spiracle, like the length, width and the area of the lobes, were almost identical in the four strains examined. The only difference in the propodeal spiracle of *Apis mellifera ligustica and Apis mellifera macedonica* was the presence of a horizontal cuticular rim on the inner part of the spiracle of *Apis mellifera macedonica*. In the prothoracic spiracle, the significance of the smaller area of the atrium, the high density of hairs and the smaller diameter of the trachea found in *Apis mellifera macedonica* was discussed as a possible mechanism for resistance to *Acarapis woodi* developed by this strain.

## INVESTIGATION ON THE FUNCTION MECHANISMS OF THE CROP AND THE OESOPHAGUS OF THE HONEYBEE

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In the honeybee, food is transferred from the mouth to the crop via the oesophagus and either is pumped further to the proventriculus, or, it may be regurgitated from the crop to the mouth via the oesophagus to be given to other honeybees, which is the function that this study attempts to elucidate. For this purpose part of the alimentary canal( oesophagus, crop, proventriculus, midgut) was isolated in a special recording chamber filled with oxygenated normal solution.

Under these conditions, observation of the spontaneous contraction of this part of the intestine indicates that the movement of the food is controlled by a system of fibers located on the walls of either the crop or oesophagus and a system of pacemaker cells generates the electrical potential which causes coordinated contractions, which can be seen under the stereoscope. The recording of the muscle action potential was achieved using fine

microelectrodes (intracellular recordings) and a specialized force-displacement transducer (the force of the contraction).

The results indicate that: a) the contraction frequency of the oesophagus is 3 Hz±0.5 (n =15) the frequency of the contraction of the crop is 0.33 Hz ± 0.09 (n=18), b) the function of the oesophagus is reversed in the isolated system (from the crop to the mouth) and c) the pacemaker activity which drives the reverse contraction is located on the anterior region of the crop.

## THE FUNCTION OF THE PROVENTRICULUS VALVE OF THE HONEY BEE *Apis mellifera macedonica*

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In the present study we examined the morphology and the physiological function of the proventriculus valve, which is located in the crop of the honey bee. In order to understand the morphology of the valve, several methods were used, such as optical microscopy and tissue staining methods. Cobaltus chloride is the dye which was used in vivo via food and toluidine blue is the dye which was applied in situ inside the crop with a microinjection system. In order to investigate the electrophysiological function, experiments in situ took place. An intracellular microelectrode was inserted in the valve and a force displacement transducer was hooked in the proximal part of the valve, in order to record the electrical and the mechanical activity of the valve respectively. The electrical signals were amplified in a DC-AC preamplifier and then were observed in digital oscilloscope. In the end the data were fed in a computer with the appropriate software for further analysis.

The conclusions from our experiments are the following: a) the morphological structure of the valve plays an important role in the filtering of the bee pollen b) the valve is a functional system with intrinsic pacemaker centers. These pacemaker centers fire action potentials and the generation of the action potential occurs independently from the central nervous system input.

## THE RACES OF GREEK HONEY BEE *APIS MELLIFERA*

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The Honey bee *Apis mellifera* L. is a social insect of great economical and ecological importance and there is a great variability among the subspecies (races) of this insect. Morphometric analysis was for a long time the only way to describe the genetic diversity of honey bees. The first studies were based on a small number of morphometric characters and then on a number of 42 characters. Recently a new method, called geometric morphometry has been developed based on the inervation of the wings of honey bee. Later on, biochemical methods have been used (allozymes – isoenzymes) to study the genetic structure of honey bees. Currently molecular markers; nuclear DNA, mitochondrial DNA and microsatellites are used to study the honey bee genetic variability.

In Greece the races of *A. mellifera* are, *A.m adami*, *A.m macedonica*, *A.m cecropia*, *A.m carnica* and in Cyprus *A.m cypria* according to morphometric analysis (Ruttner 1988). The

results of morphometric analysis of populations in Greece (Ifantidis 1979) show no hybridization among populations. Alloenzymic analysis (Badiono *et al.* 1988) of honey-bee populations in Greece shows that there was a pure race in the island of Crete and the populations of honey bees in Northern and Central Greece are distinct from the others.

Now days the genetic variability of Greek honey bees is being studied with different approaches: Classical morphometric analysis is based on the measurements of 12 morphometric characters. Phylogenetic trees are constructed by the UPGMA method using the NTSYS (Rohlf 1990) software package. Biochemical approach is based on starch gel electrophoresis of ten enzyme systems: a-GPDH, AO, MDH, ADH, LAP, SOD, ALP, ACPH, ME and EST. Results are processing using the computer program BIOSYS-1 (Swofford and Selander, 1981). Phylogenetic trees are constructed by UPGMA, Neighbor-Joining and Wagner parsimony methods using the PHYLIP (Felsenstein, 1993) software package. MtDNA approach is based on RFLP's analysis of three PCR-amplified mtDNA gene segments. The nucleotide diversity as well as the degree of nucleotide divergence between and within populations is estimated using the REAP computer package (McElroy *et al* 1991). Phylogenetic trees are constructed by the UPGMA, Neighbor-Joining and Dollo parsimony methods using the PHYLIP (Felsenstein, 1985) software package. Geometric morphometry is already in use to study the genetic variability of Greek honey bees.

The study of the genetic structure of honey bee populations in Greece and Cyprus shows that areas as Central Greece and Peloponnese seem to be hybridization zones. Using the enzymic approach it is found that the Cyprus population can be distinguished from the rest and from mtDNA analysis the Macedonian one can be. Diagnostic patterns that can be used so that the Macedonian population can be told apart from the rest are revealed. The results of enzynic and mtDNA approaches show that there is still a pure race of honey bees in isolated islands like Kasos. Greek populations can be also distinguished from Italian honey bee *A.m ligustica* with diagnostic patterns from mtDNA analysis.

The conclusion seems to be that the genetic structure of honey bee populations in this area has been changed because of migratory beekeeping and commercial breeding, during the last two decades.

The results could be useful with regard to migratory beekeeping and commercial breeding, as well as for the selection of honey bees that are more productive and resistant to enemies and diseases.

## WING DIFFERENTIATION AMONG GREEK POPULATIONS OF HONEY BEE (*Apis mellifera*): A GEOMETRIC MORPHOMETRICS ANALYSIS

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A geometric meorphometrics analysis using the coordinates of nineteen (19) landmarks located at vein intersections of the left wings was contacted on a sample of 497 bees collected from 3 mainland locations and 10 locations of Aegean islands. The ANOVA of the log of centroid sizes of wings was highly significant, though no clear geographic patterns could be identified. However, the analyses on wing shapes revealed highly significant differences between the

locations. Projections onto the first canonical plane clearly separated three locations (Rhodes, Astypalaia and Ikaria, here called Rhodes-like group) from the rest, which could be characterized as a *macedonica*-like group. Onto the first axis, specimens from Limnos lied in between these two groups. Specimens from Crete also tend to lie in the middle of the plane, but with a greater variability than Limnos ones. Apart, from Crete and Limnos specimens, no clear geographic patterns could be retrieved within the *macedonica*-like group. A neighbor-joining tree of the Mahalanobis $D^2$ distances clearly confirmed and summarized these results. The three Rhodes-like populations clearly segregated from the *macedonica*-like group. Crete specimens lied at the bottom of the *macedonica* group while Limnos specimens lied between these two main groups.

## INFLUENCE OF TROPHALLAXIS AND OF THE QUEEN ON THE DEVELOPMENT OF THE WORKER BEES AS WELL AS ON THE DEVELOPMENT OF THE BEE COLONY IN *Apis mellifera*

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In this paper we studied the influence of trophallaxis and of the queen on the development of worker bees *Apis mellifera* emerging in an incubator. The development of bee colonies established as artificial swarms from such bees was studied as well. It was found that the life span of the worker bees was not influenced by the two mentioned biotic factors. On the contrary, it seems that the royal jelly producing (hypopharingial and mandible) glands were influenced to some extent by trophallaxis with older worker bees. Nevertheless, the development of the new colonies from bees emerging in an incubator was normal, and superior when compared with colonies from normal swarms.

## *Marchalina hellenica* THE HONEYDEW PRODUCING INSECT OF PINE

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*Marchalina hellenica* (Coccina, Margarodidae), (common name 'worker') is an insect parasite of pine trees. It produces sweet secretions (honeydew), which honey bees collect and convert to honey - the pine honeydew honey. *Marchalina hellenica* is parasitic on *Pinus halepensis*, P*inus brutia*, *Pinus silvestris* and *Pinus pinea*. There is only one report of *Marchalina hellenica* on *Pinus nigra*. The insect has been observed only in countries around the Mediterranean Basin, especially in Greece, Turkey, and S. Italy. The bibliography related to the biology of the insect is limited: Nikolopoulos (1964, 1965), Marotta & Priore (1992), Fimiani & Solino (1994), Priore et al. (1997). *Marchalina hellenica* is of great importance for Greece because pine honey represents 65% of the annual honey production.

Observations made over two years (2001 and 2002) in the woodland of Seih-Sou, Thessaloniki provided the data for this study. Samples from the pine tree cuttings were taken weekly from exactly the same location. Observations were made on the biological cycle of the insect, overwintering,, the start time and duration of every ecdysis, the length of each stage of development, the mortality rate, the behaviour of adult and immature stages and fluctuations in honeydew production related to the different stages in the insects' life cycle. All of the following were continuously observed under laboratory conditions: adult insect behaviour, ovulation time, duration of ovulation, rate and total number of eggs laid, appearance of 1st instar larvae and the morphological characteristics of the adult and larval stages.

Results are summarized as follows:

- ☑ The appearance of the first adult females *Marchalina hellenica* seeking an ovulation site takes place after the 25th of March, in the Thessaloniki area
- ☑ About 78.6% of the adult females leave the old feeding site and find a new site for ovulation
- ☑ A 87.3% of the adult female *Marchalina hellenica* ovulate in the laboratory, needing no special circumstances, 2-4 days after capture
- ☑ There is a linear relationship between body weight and number of eggs laid ($R^2$ =95.32)
- ☑ Although the ovulation period in the laboratory reaches 17 days (Mean= 14.35 days), 67.7% of the eggs are laid in the first 5 days
- ☑ Average number of eggs laid is 222, although total number of eggs could reach the 400
- ☑ In the field, the average number of eggs measured in a 'cotton-wool' like nest was 184
- ☑ The average life span of the egg-laying female is 30.66 days
- ☑ In the field one can observe egg-laying female, eggs and 1st instar larvae all at the same time, in the same ovulation position for a period of about 1 month (May 9th- June 14th)
- ☑ 1st instar larvae appear on the tree branches at the beginning of May, their antennae have 6 segments and they measure 931.2 μm in length and 393.3 μm in width
- ☑ 1st instar larvae undergo 3 ecdysis. With the 3rd ecdysis which takes place at the end of October, the insect becomes a 2nd instar larva
- ☑ 2nd instar larvae have 9 antannal segments and they measure 3,673 μm in length and 1,628 μm in width
- ☑ During each ecdysis the insect can change its feeding position
- ☑ The first drops of honeydew appear around mid August; the quantity increases according to the size of the insect, while its production decreases during ecdysis. This decrease is not always noticed by the beekeepers because only a small fraction of the insects undergo ecdysis at the same time
- ☑ Heavy rain does not wash the insects off the trees or reduce honeydew production (unless it is accompanied by temperature decrease).

This research work will continue in order to throw more light on the biology of *Marchalina hellenica* and to find out more about the effect of weather conditions on honeydew secretions.

## INFECTION OF THE FIR TREE (*Abies cephalonica*) WITH THE HONEYDEW PRODUCING INSECT *Marchalina hellenica* (GENADIUS)

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Sixty 60% of the annual honey production in Greece comes from honeydew, produced by the honeydew insect *Marchalina hellenica* (Genadius) on pine trees which is the natural host of the insect. During the decade 1990-2000 many attempts were made to establish the insect on the fir tree (*Abies cephalonica*).

Finally in 1995 the establishment of *M. hellenica*, mainly in the mountains of Menalo and Helmos, was verified for the first time. The effectiveness of the establishment of the insect was 86,1% (average) in 1995 and 80.6% (average) in 1996. Honey was first produced in 1999.

## MULBERRY GENETIC RESOURCES IN BULGARIA

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Collection of mulberry genetic resources and their conservation are important to meet the different breeding programs The characterization of genetic resources is of utmost importance because it gives the idea whether the material collected is differing from existing ones. Evaluation is essential to know the genetic potentiality of mulberry varieties. Without systematic evaluation of existing germplasm the available gene pool cannot be utilized effectively. As a prerequisite to efficient utilization of germplasm, it should be properly characterized, evaluated and catalogued on the basis of useful genetic characters. Systematic documentation and exchange of information thereafter plays a very important role in the ultimate use of resources. Documentation becomes the most critical activity to make the germplasm utilization feasible through providing the descriptive information to the breeders for decision making.

In Bulgaria mulberry has grown naturally since the ancient times and now the following species are available in the country: *Morus Alba, M. bombycis, M. multicaulis M. kagayamae, M. rubra and M. nigra.* One of the main activities of Sericulture Experiment Station (SES) in Vratza has been the collection, characterization and evaluation of the indigenous and exotic mulberry varieties.

At the beginning of 20th century all the mulberry trees and plantations in Bulgaria were of the local wild variety, characterizing with excellent adaptation to the climatic and soil conditions,but having too low leaf yield. 12 mulberry varieties were introduced for the first time from Italy in 1930.After that mulberry varieties have been imported for many times from the Soviet Union (in 1956, 1958, 1965, 1967), Japan (in 1963,1970, 1974), Rumania (1964), China (1967, 1982) and Egypt (1998). As a result now there are 79 exotic mulberry accessions at the germpalsm, maintained at SES-Vratza, including 11 from Italy, 2 from Russia, 12 from Georgia, 17 from Japan, 10 from China,8 from Azerbaidjan, 4 from Ukraine etc. The number of the indigenous mulberry accessions, maintained at the station is more than 50.

For enrichment of mulberry germplasm SES-Vratza has organized several field trips for collection of accessions. The imported from Italy exotic accessions and those collected in Bulgaria until 1932 were characterized and evaluated and the varieties N3, N21, N24, N26, N101, N106, N112 were recommended for use at the field level. From indigenous accessions, collected in 1950 and 1953 were selected the varieties N 59,N51, N33 and N53 having higher leaf yield. In the period 1940-1950 were obtained several mulberry hybrids between the female varieties N24, N103, N106 and the male varieties N3, N21, N62, N112. As the best combinations were recommended: N24 x N3, N103 x N3 and N106 x N3.

During the period 1957 – 1965 the varieties N3, N24, N59, N101, N106, Adreuli, Pobeda, Tbilisuri and Kokuso 70 have been tested in the main sericulture regions of Bulgaria. The varieties N106, Kokuso 70,Pobeda and Tbilisuri gave the best results and were approved by the government for commercial saplings production. In the period 1967-1977 were investigated the hybrid offsprings of the varieties Georgia,Kinriu,Kokuso 20,Kokuso 27,N24 and N106 under natural pollination. It was detected that the offsprings of Georgia, Kinriu and N24 had the biggest percentage unlobed leaves- 89% , 67 % and 63 % respectively. During the period 1976-1978 the mulberry varieties Kokuso 21, Kokuso 27 and Kinriu, introduced from Japan and the selected at SES-Vratza new varieties Vratza 1 and Vratza 18 were tested and approved for commercial use.

New mulberry accessions were created by using the methods of experimental polyploidy and hybridization. Penkov (1980) made a hybridization between the varieties N3, N116, N118, and N120 belonging to the species *M.alba, M.kagayamae and M. latifolia*. High percentage of trees with unlobed leaves manifested the hybrids N24 x N116 – 90 % , N117 x N3 – 85 %, and N119 x N3 – 75%.These hybrids were approved by the government as hybrid varieties (Hybrid 50,Hybrid 78 and Hybrid 96) for commercial use in 1980 .

The research work completed in 1981 proved that the F1 mulberry offsprings had very high variation in leaf lobation type and the mother variety influenced in bigger extent the leaf lobation type in the progeny. The leaf size was inherited intermediately in F1. In 1995 a methodology for characterization and evaluation of mulberry accessions was developed by Petkov (1998). Now the research work in mulberry selection is considered as very important activity in SES-Vratza. It is necessary to continue the collection of new mulberry varieties both exotic and indigenous. In the recent years more than 2500 hybrid seedlings were obtained, planted and now they are under evaluation. The future direction of the breeding work should be the so called “far” hybridization between varieties from different geographical regions, different species and especially between varieties having different chromosome number.

As a result of the hard breeding work at SES-Vratza were selected the highly productive mulberry varieties Vratza 1,Vezletz and Vratza 18 which could be of interest for introduction in other countries as well.

## BEEKEEPING PLANTS

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About a 100 Greek plants which are important in beekeeping are included in this report, which was compiled over a period of eight years. The data file on each plant includes: the classification and description of the plant, a few beekeeping comments and five pictures of the plant. It was very important to include a picture of a bee ‘working’ on the flower of each plant so that it could be characterized as a plant used by bees..

The pollen carried by the bee is also shown in the pictures. This report could be a very helpful tool for recording the plants used by bees in Greece and for identifying the pollen collected by the bees.

# THE CHARACTERISTICS OF GREEK UNIFLORAL HONEYS

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In a collaborative study of the characteristics of unifloral Greek honeys, research was carried out into their volatile substances (the aroma), their microscopic characteristics, their antioxidant and antibacterial properties, their calorific value and their osmophlic yeast flora. Greek honeys from pine and fir honeydew, thyme, chestnut and cotton, heather, orange and sunflower were studied. In total 30 different physicochemical characteristics were examined, including sugars, minerals and enzymes, proline and all the constituents mentioned in the EEC honey directive. The following factors were found to be important in identifying the floral origin of the honey: the electrical conductivity, the total mineral content, the amount of glucose and fructose, the reduced sugars, the maltose and arabinoze and the microscopic characteristics. As well as the physicochemical characteristics, 177 volatile substances were identified, of which the following were found to be floral specific:

*a)* A-pinene, borneol and high concentrations of nonanal, are characteristic of pine and fir honeydew honeys. Particularly in the fir honey a number of benzene isomers with 3 C and 4 C appear

*b)* Nonyl ester acetic acid and high concentrations of 1,2 dichloro-benzene are characteristic of chestnut honey

*c)* Benzonitrile, 1-phenylbutan-2,3-dione, 2- hydroxy-benzaldehyde and high concentrations of phenyl acetaldehyde are characteristic of thyme honey

*d)* Isophorone, 2-hydroxy-3,5,5-thimethyl-1-cyclohex-2-enone, 2,6,6-thrimethyl-1,3-cyclohexadiene-1-carboxaldehyde, 3,3,3 thrimethyl- cyclohexene, are characteristic of erica honey

*e)* Beta germagrene and gamma selinene, are characteristic of sunflower honey

*f)* 3-methyl-1-hexanol, 3,4-dimethyl-1-pentanol, benzopropanal, are characteristic of cotton honey

*g)* Lilac aldehyde isomers, 3,4-dimethyl-1,6-Octadien-3-ol, 2,6-dimethyl-(Z)-1,6-Octadiene are characteristic of citrus honey

*h)* Methylphenyl-ethanone isomers, camphor, 2,6,6,9-tetramethyl- cyclo[5,4,0,02,8] undec-9-ene and 2,3,4,7,8,8a-hexahydro-3,6,8,8 tertramethyl-1H-3A-Methanoazulene are characteristic of sage honey.

All analyzed honeys have shown antioxidant and antibacterial activity, although honeys from different floral sources and geographic regions have shown great variability in antioxidant and antibacterial activity. Ferric Reducing Antioxidant Power values ranged from 115 μM (citrus honey) to 990 μM (Fir honey). The calorific values ranged from 3080 (pine honey) to 3755 Kcal/Kg (sunflower honey).

## CONTRIBUTION TO THE IDENTIFICATION OF GREEK HONEY

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Samples of Greek honey have been analysed for their sugar and phenolic profiles, the volatile compounds, moisture and electrical conductivity. The possibility of a correlation of these characteristics with the botanical and/or geographical origin is being investigated. Most samples were collected from beekeepers of Crete in 2001.

## CHEMICAL ANALYSES OF GREEK BEE-HONEYS – BIOLOGICAL ACTIVITIES

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Greek honey has been famous since ancient times, and now there is a renewed and increasing interest in using it as part of the traditional Mediterranean diet. This report is one part of the systematic research into the chemical composition and biological properties of this celebrated natural product. In this study we report the detection and isolation of 3-hydroxy-4-phenyl-2-butanone in five out of forty Greek honeys. This hydroxyketone has never been detected in any kind of bee-honeys before - it has been found naturally only in wine and among the flower volatiles of *Wisteria floribunda* (Fuji) from Japan, and *Mimusops elengi* found in Malaysia. Forty Greek mono- and unifloral bee-honeys samples produced in thirty different regions of Greece, were studied for their volatile secondary metabolites. In five, out of the six *Thymus* bee-honeys examined samples, (67.74 and 72.29% in *Thymus*) , a trihydroxy ketone was detected, ranges from from 12.98-68.29%. The compound has been isolated and identified by modern spectral means (1D, 2D NMR). This triol has been shown to be unique to samples with the higher percentages of *Thymus sp*. and thus may serve as proof of the floral source of these honeys.

The antimicrobial activities of samples as well as the pure isolated compound were determined, using the dilution technique, by measuring their MIC, against two Gram-positive bacteria: *S. aureus* and *S. epidermidis*, four Gram-negative bacteria: *E. coli*, *E. cloacae*, *K. pneumoniae* and *P.aeruginosa* and three pathogenic fungi *Candida albicans*, *Candida tropicalis* and *Candida glabrata* all of them strains of ATCC.

Through the antimicrobial screening, the bee-honeys as well the characteristic hydroxyketone, proved to be significantly active against all nine tested microorganisms (MIC values 0.04-3.2mg/mL). The results of our studies suggest that the activity of the studied bee-honeys can be attributed to the existence of the isolated hydroxyketone, as well as to the triol, which appeared to possess strong activities against all tested microbia. Since this compound appears to occur only in thyme honeys, it can be used to prove the floral source of these honeys, which command top prices in the Greek honey market.

## ANALYSIS OF HONEY SAMPLES WITH ISOTOPE RATIO MASS SPECTROMETRY IN THE LABORATORY OF D' CHEMICAL SERVICES OF ATHENS OF THE GENERAL CHEMICAL STATE LABORATORY

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The adulteration of honey with isoglucose or other syrups derived from corn can be detected by the isotope ratio mass spectrometry. The use of an "internal standard" with the analysis of honey proteins means that all types of honey can be tested accurately for corn syrups.

The vast majority of honey samples from Greek producers examined in the laboratory of D' Chemical Services of Athens of the General Chemical State Laboratory were free from added syrups.

## ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF GREEK HONEY

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Honey has been known since ancient times for its therapeutic and medical uses. It has been used as a medicine for thousands of years and its curative properties are well documented. Honey possesses antioxidant and antibacterial substances that inhibit growth of a wide range of bacteria and fungi, which cause various abscesses and surgical infections. It is a 'traditional' remedy for the treatment of infected wounds, which has recently been rediscovered by the medical profession, particularly where conventional modern therapeutic agents are failing.

Oxidative stress, defined as an imbalance between free radical production and antioxidant defense system favoring oxidation, plays a significant role in the development of chronic diseases. Free radicals and reactive oxygen species have been implicated in contributing to ageing and to many disease states including cancer and cardiovascular diseases. Humans protect themselves from reactive oxygen species, in part, by consuming dietary antioxidants. Honey is an agricultural product that can be a source of antioxidants.

The antibacterial activity of honey had been attributed to: a) the osmotic effect, whereby water is drawn away from the microorganisms reducing their ability to survive b) acidity, its pH being between 3.2 and 4.5, and mainly to c) hydrogen peroxide, which is produced enzymically in the honey by the bee. Recent studies have shown that honey has phytochemical antibacterial components, which are believed to be the main non-peroxide antimicrobial factors.

Honey from three floral sources (spruce, orange and thymus) and from different geographic regions of Greece, were characterized for their antioxidant and antibacterial activity. Antioxidant capacities of honey were determined using the FRAP assay (Ferric Reducing Antioxidant Power), while their growth inhibition against two phytopathogenic bacteria *Pseudomonas syringae* and *Bacillus subtilis* were determined using the broth dilution method. Specifically the minimum inhibition (MICs) and bactericidal concentrations (MBCs) of honeys have been estimated.

In this study all analyzed honeys have shown antioxidant and antibacterial activity. FRAP values ranged from 115 μM to 990 μM, while MICs values have been estimated for spruce and thymus in the range of 10% to 20% (w/v) of honey solutions and MBCs values in the range of 12% to 25%, while for orange MICs values have been estimated in the range of 15%-25% and

MBCs values >25%. Furthermore darker colored honey (spruce) contained higher antioxidant power than lighter honey (orange). The bacteria specie *Bacillus subtilis* was more sensitive than *Pseudomonas syringae*. Honey from different floral sources and geographic regions have shown great variability in antioxidant and antibacterial activity.

## ULTRASOUND-ASSISTED ISOLATION OF THE VOLATILE COMPOUNDS FROM SOME UNIFLORAL GREEK HONEYS

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The volatile fraction of four unifloral Greek honeys (orange, eucalyptus, chestnut and cotton) was studied. A new procedure for isolating the volatile compounds from honey was developed, employing ultrasounds to assist solvent extraction. This procedure does not require heat, thus no thermally generated artifacts are produced. Furthermore, it is quite rapid, easily carried out and does not require special equipment.

The extraction was followed by GC/MS analysis. Certain volatile compounds seem to be characteristic for each unifloral honey, depending on the botanical source it comes from. In most cases, correlation between the honey extract and the flower extract of the corresponding botanical source is adequate. Linalool derivatives predominate in the orange honey extract, with (*E*)-2,6-dimethyl-2,7-octadien-1,6-diol being the most abundant, while in the citrus flower extract linalool predominates.

Chestnut honey is characterised by the presence of 1-phenylethyl alcohol, a compound also found in the flower extract. Compounds possessing "3,5,5-trimethyl-2-cyclohexen-" skeletons were the most abundant in eucalyptus honey. Moreover, exo-2-hydroxycineole is present only in eucalyptus honey, while 1,8-cineole (eucalyptol) predominates in the flower extract. Finally, the extract of cotton honey consists of an array of sikimate-pathway derived aromatic compounds, with phenylacetic acid being the major constituent.

## A METHOD THAT RECOGNIZES POLLEN GRAINS OF DIFFERENT SPECIES WITHIN THE ROSACEAE AND LAMIACEAE FAMILIES

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Pollen grains from melliferous plant species of the Rosaceae and Lamiaceae families were photographed under the optical microscope with a digital camera and their dimensional characteristics were measured using an appropriate software programme. For the 10 Rosaceae species examined, the opening (length) of the germ pore, median, circumference and area, were measured, and for the 8 Labiateae species, the length, width, circumference and area of pollen grains were measured.

The statistical analysis of data collected showed that certain measurable pollen grain characteristics alone, or in combination, can be used to distinguish the different plant species. In addition, if the differences between species in the measured pollen grain characteristics are

small, it is possible to combine them with morphological characteristics to increase the degree of accuracy of plant identification.

This methodology can be applied to identify the plant origin of bee honey.

## DETERMINATION OF GEOGRAPHICAL ORIGIN OF THYME HONEY BASED ON VOLATILE COMPONENTS

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The type and concentration of the volatile components of honey are very important for the determination of its botanical and geographical origin. The "aromatic profile" of honey differs according to its floral origin, but there can also be variation in honeys from the same flowers but with a different geographical origin. It is important to be able to determine the country where honey has been produced, and the new European Community Directive for honey (2001/110/EEC) requires that the country of origin is recorded on the product label.

The constituents of honey, such as amino acids, flavonoids, pollen spectrum and volatile components have been used to determine the geographical origin. In this work samples of thyme honey from five different regions of Greece have been analyzed to identify differences between them. The results indicate that differences exist which are due to the unique flora of each region. The research is continuing with more samples, so that the characteristic compound-markers for the geographic origin of Greek thyme honey can be established.

## DETERMINATION OF ADULTERATION WITH IMPORTED HONEYS THROUGH POLLEN ANALYSIS

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It is very difficult to detect if Greek honey has been adulterated with imported honey by classical chemical analytical methods. However, The country of origin can be found out by the use of melissopalynological analysis. Pollen analysis can be used in order to determine not only the country of origin but also mixtures of honeys from different countries. In this study we have tried to separate categories of honey with the same botanical origin from different countries. We have analyzed 68 samples of imported honey. Orange honey from Spain, Turkey, Italy and Argentina, pine honey from Turkey, forest honey from Spain and Italy and finally thyme honey from Spain, Italy and Turkey. The results of **t**he pollen analysis were compared with Greek samples. We tried to detect specific combinations of pollen grains or other characteristics, which could separate the Greek from imported honeys.

The imported honeys have different percentages of the predominant pollen as well as of the secondary pollen grains. Imported Citrus honey shows higher percentages of Citrus type pollen(>10%) in comparison with Greek honeys and also has secondary pollen grain which does not appear in Greek orange honeys. Thyme honeys from Italy and Spain appear to be from a different botanical type of Thyme than the Greek ones while Turkish thyme honey has characteristic combinations of secondary pollen grains. The Turkish pine honey can be

separated from Greek because of certain pollen grains while the forest honey from Italy and Spain can be identified by their honeydew elements. In each case the pollen analysis must be performed in combination with sensory analysis. The enforcement of new honey directive of E.E. (2001/110EC) and its implications for Greek honey production and trading was discussed.

## THE ENFORCEMENT OF THE COUNCIL DIRECTIVE (20001/110EC) RELATING TO HONEY, AND ITS IMPLICATIONS FOR GREEK HONEY PRODUCTION

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The enforcement of new honey directive of E.E. (2001/110EC) and its implications for Greek honey production and trading are discussed. Beekeepers and distributors should be made acquainted with problems that they may face with the new composition criteria, especially with the amount of glucose and fructose in combination with the electrical conductivity.

Is not unusual for Greek blossom honeys to contain a proportion of honeydew honeys (pine and fir honeys) or chestnut honey. This might happen naturally when the bees are collecting nectar, or technically when beekeepers and distributors blend the honeys in order to slow down the speed of crystallization. This means that the honey may not be pure by the EEC criteria, and care must be taken to label the final product according to the EEC regulations.

## MELISSOPALYNOLOGICAL CHARACTERISTICS OF THE MAIN TYPES OF GREEK UNIFLORAL HONEYS

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The present study was carried out within the framework of the National Programme for the Improvement of Honey Quality and Trade, based on EU Regulation 1221/97. The objective of the study was to investigate the microscopical characteristics of the main types of Greek unifloral honeys, so as to contribute to their identification. The analyses performed involved organoleptic evaluation, melissopalynological analysis (qualitative and quantitative), as well as measurement of some physico-chemical parameters of diagnostic value as electrical conductivity and color. The analyses were carried out according to the official methodology.

Statistical analysis of the results showed the melissopalynological characteristics of the main types of Greek unifloral honeys, that is, the percentage of main pollen and the HDE/P ratio for nectar and honeydew honeys, respectively, the total number of plant elements in 10g, and frequencies of all pollen grains found in the sediment. It is expected that further study of the results obtained will give tools useful towards the differentiation of Greek unifloral honeys according to their geographical origin, and/or their differentiation from relative honeys produced in other countries.

## SELECTION OF SOURCE SILKWORM (*Bombyx mori* L.) LINES, GENETICALLY MARKED BY SEX, FOR COMMERCIAL HYBRIDIZATION

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A study was made on the results from mathematical prediction in the selection of 6 *Bombyx mori* L. lines, genetically marked by sex at the egg- and larval stages, for commercial hybridization.

It was established that the statistic-genetic model used, applying selection indices, was suitable for mathematical prediction in the selection of silkworm lines, genetically marked by sex, for producing commercial hybrids. Among the great number of combinations, it enabled the selection of the best lines for commercial use, thus reducing considerably the costs and increasing the efficiency of experimental and breeding activities.

## COMPARATIVE PERFORMANCE OF FOUR CHINESE SILKWORM HYBRIDS UNDER SPRING AND AUTUMN REARING IN NORTHERN GREECE

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Four of the popular Chinese silkworm hybrids, namely SUJU X MINHU, MINHU X SUJUE, 871 X 872 and 872 X 871, suitable for both spring and autumn rearing, were reared during spring and autumn of the years 1999 and 2000 at the Komotini Agricultural Research Station in Greece under the local climatic conditions. All hybrids were fed with leaves of the Japanese Kinriu mulberry, grown locally at the Komotini Research Station. Their performance, throughout the rearing period, was recorded with reference to food consumption during the fourth and fifth instar, larval stage duration, mounting percentage, raw and dry cocoon weight, reelable cocoon percentage, cocoon silk ratio and estimated total yields of raw and dry cocoon and raw silk per box of eggs.

The studies discovered a remarkably higher feed intake by all hybrids on spring rearing followed by higher production parameters, such as mounting percentage, raw and dry cocoon weight and cocoon silk ratio. Slight differences were observed between the hybrids within the parameters researched.

In both spring and autumn rearing hybrid MINHU X SUJUE showed a higher food consumption, connected to longer larval stage duration and higher raw and dry reelable cocoon yields. Cocoon silk ratio in all cases did not appear linked to the other parameters and hybrid MINHU X SUJUE showed higher percentages in both spring and autumn rearing.

In general all hybrids showed a good adaptation to the local environment, both for spring and autumn rearing, with the hybrid MINHU X SUJUE giving the best performance in spring rearing and with an almost uniform performance of all hybrids in autumn rearing. All the observed differences in the hybrids performance were within the internationally accepted standards.

## SILKWORM (*Bombyx mori*) GENE BANK MAINTENANCE AT KOMOTINI AGRIC. RES. STATION – SELECTION AND IMPROVEMENT OF PURE LINES

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The silkworm is very common and is widely spread throughout the world. It can live in a wide variety of environments, and this, combined with the great length of time that it has existed in each environment, has created a huge variety of genotypes, and also a large variation in productiveness. This gives scientists plenty of material to work with for producing genotypes suitable for each specific environment and for each specific goal, so ensuring that the silkworms will perform better than others imported from other environments. This is why the Komotini Agricultural Research Station is developing a silkworm gene bank. A reasonable number of pure lines of Japanese and Chinese type silkworm are being kept.

Research is being carried out into their main heritable economical traits. At the same time they are being selected and improved and their potential for use in the creation of high producing hybrids, which are well adapted to the local environment is being investigated. Komotini Agricultural Research Station was provided with the pure lines by sericulural institutions abroad with which they are cooperating. Up until now the all the lines have adapted very well to the local environment, performed satisfactorily and shown high improvement potential.

## CENTER FOR CONSERVATION AND PRODUCTION OF MULBERRY VARIETIES FOR THE DEVELOPMENT OF SERICULTURE IN GREECE

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In the past, sericulture made an important contribution to the rural economy, and now it has a part to play again. Today, in Greece, it is very important that agricultural land is used wisely and productively so that the farmers can survive and thus depopulation of rural areas can be slowed down. To this end sericulture could provide an important additional income for families for two or three months of the year using existing equipment and installations.

To start sericulture in an area you need to grow mulberry trees. These play an important multiple role in an area because they also provide food for domestic animals. Only the leaves are needed to feed the silkworms, but the mulberry tree has many other uses: it also gives fruit, which can be used in medicine; it provides fire wood and can be used in the production of paper. It is also used in fire protection by creating fire protection zones.

The Sericulture Laboratory, on the campus of the Agricultural University of Athens has been in operation since 1920. In addition to its other work, this department oversees the selection of the mulberry varieties most suitable for silkworm rearing, easy maintenance and cultivation. In 1/3 hectare of land the Lab maintains 20 of the best mulberry varieties in the world. Characteristics that are studied are: the adaptation of the varieties to Greek climatic conditions, resistance to different diseases, adaptation to different soil types, quantity and quality of the leaves produced.

In cooperation with two nurseries we produce 80,000 to 100,000 trees every year so that new mulberry plantations can be established in different areas of Greece. We are also trying to produce trees from local mulberry varieties that have existed for several centuries in those

islands, which have traditionally been involved in silk production. New trees are grown either from cuttings or from seeds.

## INVESTIGATION ONTO THE EFFECTIVENESS AND TOXICITY OF LACTIC ACID WATER SOLUTIONS

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In order to investigate the possibility of an effective, safe and economic use of lactic acid in the control of Varroa mite, a series of experiments took place from October 1999 till December 2001. Two different water solutions of the acid were used: 15% and 5%. The solutions were applied in different seasons and temperatures, and in colonies with different populations and brood area.

The results showed that:

- The 15% solution is very effective- up to 96.6%, but the 5% solution is not very effective (52%) when applied to colonies without brood
- Effectiveness is higher in lower temperatures, and varies with the area of brood. Lactic acid is effective only on the Varroa carried by adult bees
- In colonies which rear a significant amount of brood, 6 applications, one every 3 days, are needed
- No residues were detected after 24 hours
- 15% and 5% solutions of lactic acid are not toxic to adult honey bees
- When 15% solution is applied at temperatures above $18^0$C and in colonies which rear brood, it has side effects on the biological rhythm of the colonies: egg laying is reduced or stops altogether, loss of the queen (50%), reduced ability to produce new queens, reduction in the number of hatching eggs
- The 5% solution give better results when applied late in Autumn and has no effect on bees.

We conclude that lactic acid solutions are harmful to bees and are not to be used for Varroa control.

## FIELD STUDY TO ASSESS THE EFFECTIVENESS AND SAFETY OF APIGUARD®

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To assess the efficacy of APIGUARD®in controlling Varroa mites, two experiments were conducted on colonies of *Apis mellifera macedonica* during the Spring and Summer of 2002. In the first experiment, APIGUARD® was applied to two groups of infected colonies in the following ways: Group 1 -Ten colonies were treated with 2x50g (in aluminum trays) at a two week intervals, and Group 2 -Ten colonies were treated with 2x50g (spread on a 10x10 cm flat

support) at two week intervals. To assess the possible influences of APIGUARD® on honeybees, ten untreated colonies were kept as a control group. The efficacy of APIGUARD® on Group 1 was 89,29% and on Group 2 was 93,37%.

In the second experiment, APIGUARD® was applied to three groups of infected colonies in the following ways: Group I -Ten colonies treated with 2x50g (in aluminum trays) at two week intervals; Group 2 -Ten colonies treated with 2x50g (spread on a 10x10 cm flat support) at two week intervals and Group 3 -Ten colonies treated with 2x25g (spread on a 10x10 cm flat support) at one week intervals. The efficacy of APIGUARD® on Group1 was 95,09%, on Group2 was 96,59% and on Group3 was 94,95%.

In addition, the growth of colonies (population and brood frames) and their behavior was observed and compared with control colonies during the two experiments. The growth of colonies was normal and no adverse changes in bee behavior such as aggressiveness, robbing or erratic movements were observed.

## INVESTIGATION ONTO THE EFFECTIVENESS AND TOXICITY OF APILIFE-VAR FORMULATION

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The effectiveness and toxicity for honey bees of Apilife-VAR formulation were investigated under different environmental conditions on honey bee colonies rearing different areas of brood. The 1st experiment took place at a temperature below $18^0$C. Two pieces of the formulation were applied, at a rate of one piece every 12 days, to colonies with populations covering 10 frames and an area of 3-8 $dm^2$ sealed brood. The 2nd experiment took place at a temperature above $18^0$C. Four pieces of the formulation were applied at a rate of two every 12 days, to colonies with populations covering 8-10 frames and an area of 17-24 $dm^2$ sealed brood.

The results showed that the effectiveness of Apilife-VAR formulation is 95.2% when the average temperature is higher that $18^0$C, the application lasts for 24 days and two pieces of the formulation are used every 12 days, while it is 90.7% when the application lasts 12 days under the same conditions. The effectiveness of the formulation is 83.1% when average temperature is lower than $18^0$C, the application lasts for 24 days but only one piece of the formulation is used every 12 days. At the above temperatures, toxicity for the honeybees was minimal, and no other effects were observed on the biology of the colonies.

## CONTROLING VARROA MITES WITH COUMAPHOS BEE STRIPS 10%

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In order to provide a consistent control of varroosis without harmful effects on adult bees and brood, treatment with coumaphos bee strips 10% (Bayer) was tested in a field trial. Twenty-

four colonies of *Apis mellifera Macedonica* which were naturally infested by Varroa mites (*Varroa destructor*), were examined thoroughly and subsequently divided into two groups of 12 colonies each, A and B, according to their population, area of brood and Varroa infestation. In October 1st 2001, two strips were placed between the 3rd and 7th frame of the nest area of each colony. The A type strips (Coumaphos 10 % Bee Strips) were placed in colony-group A and likewise B strips (Coumaphos Placebo Bee Strips) in B group. The number of dead bees and dead mites for each colony was counted every 1 –3 days till the end of treatment period (November 11th). The effectiveness of the treatment was calculated by the ratio of mites killed during the experiment to the total number of mites present in the colony. The safety of the test product, was assessed by counting the dead bees in front of each colony every day, by inspecting the brood patterns and queens present every 7 days, by counting the frames covered by bees to estimate the bee population and by observing any adverse effect on bee behavior such as aggressiveness, robbing, erratic movements etc.

The efficacy of Coumaphos 10 % Bee Strips after 41 days treatment was high and reached the average of 98.6±4.8. The majority of the mites were dropped during the first 24 days (about 97.6% of the mites were killed). The number of dead or moribund bees that were counted at colony entrances was negligible, average 1,04 bee/day in colonies treated with Coumaphos 10 % Bee Strips and 0.66 in colonies treated with Placebo. Differences were not significant ($p<0.005$). Brood patterns during the four inspections (8/10, 15/10, 22/10, 29/10) were found compact in all colonies and no replacement of queens, no irregularities in laying eggs or "pepperbox" symptom (a mixture of capped and uncapped brood cells) were observed. The appearance of sealed and unsealed brood reflects physiological condition. The strips had no repellent effect on bees. Flight activity was normal and no adverse effects on bee behavior, such as aggressiveness, robbing, erratic movements or crawling of bees near the hive entrance were observed.

## ADHESION MECHANISMS OF VARROA DESTRUCTOR ONTO *Apis mellifera macedonica*

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Because the mechanism by which the Varroa mite adheres to the honey bee could be the key to the biological control of the mite, a scanning electron microscope was used to study the ambulacra of *Varroa destructor* in Greece. The use of negative pressure to dislodge Varroa from *Apis mellifera macedonica* was also investigated.

The results confirmed that the ambulacra of Varroa have a sclerotized structure while at the same time they have the ability to expand and form a sucker-like structure. Negative pressure of 2 Bars did not have a significant effect in dislodging Varroa from bee bodies.

## CONTROL OF VARROA MITE IN NACED WINTER CLUSTERS

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The procedure for creating artificial winter clusters (package bees) in *Apis mellifera* and their temporary establishment in properly adapted and preferably plastic containers is described in this paper.

Data is given concerning control of varroa mite by applying different acaricides on the clusters, always outside the hives. Suggestions are also made about ways of controlling infectious diseases in the new bee colony which such winter clusters may cause. Finally, the advantages and disadvantages of the new technique are discussed.

## ASSESSMENT OF THE CARDIOTOXIC ACTION OF PESTICIDES ON INSECTS, USING PHYSIOLOGICAL TISSUE-PREPARATIONS BASED ON THE ISOLATED HEARTS OF *Apis mellifera macedonica* AND *Tenebrio molitor*

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Pesticides are one of the major problems for apiculture. Honeybees, non-target insects, are not only directly exposed to these compounds in the environment, but also pesticides are used inside the beehive to fight parasites.

In our studies for the assessment of the cardiotoxic action of pesticides, the isolated heart of the honey bee was placed in a recording chamber filled with oxygenated physiological solution (saline). The power and the frequency of the heart contraction were monitored using a specialized force displacement transducer. To monitor intracellular activity from the cardiac pacemakers glass floating microelectrodes were used. Both the mechanical and electrical activity was fed to a computer through a data acquisition computer system for further analysis of the response of the tissue-preparation in the presence of normal saline (control) and in the presence of saline where the pesticides (imidacloprid, 2,4-D, deltamethrin at different concentrations) were diluted. For the validation of the toxicity the no effect concentration (NoEC) of a pesticide, the minimum concentration of the pesticide diluted in physiological saline, which has no significant effects on the parameters of the cardiac contraction, was estimated.

## ASSESSMENT OF THE ACTION OF THE INSECTICIDE IMIDACLOPRID (GAUCHO) ON THE CENTRAL NERVE CORD OF THE INSECT *Tenebrio molitor* AND THE HONEYBEE *Apis mellifera macedonica*

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In insects the respiratory activity includes the phase of inhalation and the phase of exhalation. In most species, the expiration begins with the contracture of all lateral (dorsal-abdominal) muscles. The synchronization of all muscles, which belong in different segments, is

controlled by a system of interneurons in the central nervous system (CNS), which is called central pattern generator (CPG). These interneurons form cholinergic synaptic terminals with the respiratory motoneurons. In this study, using a variety of electrophysiological methods, it was possible to investigate the modification of the respiratory rhythm of the beetle *Tenebrio molitor* and the honeybee *Apis mellifera macedonica*, during the perfusion of the isolated nerve cords with the neonicotinic insecticide Imidacloprid. Extracellular records were obtained from motor branches of the isolated nerve cords, using a modified suction electrode.

Because of the molecular structure, the size and the polarization that Imidacloprid presents, it binds on the molecular receptors which normal bind acetylcholine (Ach), causing the block of the Ach receptors. This binding activates the nicotinic receptors. However Imidacloprid is not hydrolyzed by Acetylcholinesterase causing continuous activation of nicotinic receptors. As a result overexcitation of the cholinergic synapses, spasms and at last the death of the insect occur (Mullins 1993). For the validation of the neurotoxicity induced by Imidacloprid, the no effective concentration was estimated for both in vitro preparations.

## CONTROL OF THE HONEY BEE MITE, *Varroa destructor* WITH ESSENTIAL OILS AND AROMATIC PLANTS FROM THE GREEK FLORA

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Sixteen essential oils were examined in laboratory conditions, for their efficiency in controlling *Varroa destructor*, as well as for their possible toxicity on honey bees. Only mature honey bees infected with mites were used in these experiments. Certain essential oils (oregano, pine) were absolutely harmless to both varroa and honey bee. On the other hand, others (wormwood, eucalyptus) were able to control the mite but were very toxic to the bees at the same time. Others (juniper, laurel, peppermint, spearmint, myrtle, rosmarinus and savory) were quite efficent in the control of the mite (>75%) but also relatively toxic to the honey bees (>10%). Finally, a small number of essential oils (thyme, lavender, mild sage and wild sage) were lethal to varroa and had no effect of the viability of the bees.

Essential oils from the last group were used for testing on the activity in bee hives, in real conditions in the field. After two applications of essential oils within a month, and consequent use of apistan, we found out that the essential oil treatments had no significant effect. Nevertheless, the difference between laboratory and field results must have an explanation. Indeed, we observed a very high negative correlation between the initial mite infestation and brood surface. It is evident the presence of brood is a factor that has not been considered in the present bee hive experiment, and it is clear that it plays a very important role in the development of the mite population. From the essential oils examined, the compounds that showed the highest activity were: p-cymene, γ-terpinene, thymol, carvacrol, 1,8-cineole and thujones.

We also checked the honey produced for contamination with essential oil compounds, and we found none.

## THE USE OF ESSENTIAL OILS AS TREATMENT AGAINST THE MITE *Varroa destructor*

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The mite *Varroa destructor* is a very serious problem every year for worldwide. Nowadays the infection is treated by the use of synthetic chemical miticides. Unfortunately the use of these miticides may make parasite less vulnerable to chemical treatment. Also, these treatments may have a deleterious effect on the quality of the honey products.

In this study thymol was the main component of many different treatments in vitro and in vivo. The experiments were carried out in 2001 and 2002. The results of the best treatments were:

1. Crystal-thymol : 89% - 94,3% effective
2. Crystal-thymol in gel : 89,4% - 96,8% effective
3. Crystal-thymol plus eucalyptus oil and clove oil in gel: 73,9% - 88,6% effective.

Asuntol solution was used as control agent. The above results prove the importance of thymol against the mite infection.

## STUDIES ON A) THE BENEFICIAL ACAROFAUNA AND MICROFLORA AND B) HAEMOCYANIN INHIBITORS AGAINST VARROA DISEASE

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Studies on mites associated with beehives in Greece revealed the presence of many species, of various feeding habits (predatory, saprophagous, parasitic). With the aim of using several of those species as bio-control agents against *Varroa destructor* the predators *Typhlodromus cotoneastri* and *Cheyletus malaccensis* were reared. Biological parameters of a third predatory species: *Typhlodromus foenilis* were studied as well.

A study of natural microflora of *V. destructor* showed the presence of 16 bacterial, 2 yeast and 10 fungi strains. The survival of *V. destructor* populations has been examined in the presence of two of those bacterial strains.

In studies on the use of various haemocyanin inhibitors against *V. Destructor*, Cupric Gluconate showed promising results. This compound, given to bees in sucrose syrups, had no adverse side-effects on honey bees, even at doses of 12,5 gr Cupric Gluconate per hive.

## STUDY ON THE DEVELOPMENT OF THE POPULATION OF V*arroa destructor* (ACARI: VARROIDAE) IN COLONIES OF THE HONE YBEE *Apis mellifera macedonica* (HYMENOPTERA:APIDAE)

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The development of the population of the acari *Varroa destructor* in colonies of the honeybee *Apis mellifera macedonica* was evaluated for two years, from April 1999 to April 2001. The conclusions we have reached are based on a combination of the data collected from the study of the development of a) the adult bees' population and worker sealed brood and b) of the relative infestation rates. The development of the population of the mites found on adult bees and within worker cells has been examined at 21-day intervals, except during winter, when data recording was sparse. The average number derives from 12 colonies following a statistical analysis of the results.

The population of the mites which are found on adult bees has shown a gradual increase from May, 43 ± 25 (±SE) mites, up to the ten last days of November when the highest value of the whole research was recorded, 2991 ± 331 (±SE). Subsequently, there was a consecutive decrease up to late-March, an increase from April to late-September, 2751 ±582 (±SE) and a decrease up to the last period of calculation, 348 ± 57 (±SE) mites. In worker sealed brood, the mite's population showed an increase from April 114 ± 55 (± SE) to mid-October 2129 ± 427 (±SE). After October, the number of mites decreased to reach nil values in mid-February, while an increase was recorded from late-March to mid-August when the highest value of the whole research was recorded 3847 ± 564 (±SE) mites. Consecutively, we have observed a gradual decrease up to late January while there was an increase both in mid-February and during the last calculation which took place towards mid-March, 147 ± 46 (±SE) mites.

The effect of various colony-related parameters on the mite's population development is discussed. We hope that the conclusions concerning population development will contribute to confronting the disease in a more accurate and effective way.

## AN ALTERNATIVE PROCEDURE FOR DETERMINING THE INFESTATION LEVEL OF WORKER BEE BROOD OF *Apis mellifera* L. BY THE MITE *Varroa destructor*

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Two alternative procedures for making pieces of sealed worker brood comb bee and/or varroa proof are described in this paper. When the post-embryonic development of the host is complete, infected brood cells can be recognized by the presence of traces of the parasite (excrement, dead parasites, or the shed skins)

The "sandwich" method is compared with other previously known methods with reference to: the feasibility of large scale sampling, the accuracy with which the level of infestation by the varroa mite can be determined. The variety of information about different factors connected with the level of infestation with varroa in the hive.

## COLONIES OF *Apis mellifera macedonica* (RUTTNER) RESISTANT TO *Varroa destructor*

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In an attempt to assess the possibility of existence *of Apis mellifera macedonica* colonies resistant to *Varroa destructor*, the population dynamics of the mite were investigated. The total number of Varroa mites and their fall on the hive floor rates were assayed, in three groups A, B, C, of 12 bee colonies, from October 1st to December 20th 2001. These groups were created from three different apiaries, in each of which a different treatment regime against varroosis was applied.

The total numbers of adult mites in each hive ranged from 302 to 4590. Statistical analysis of data showed no significant differences in the recorded mite numbers (mean ± standard deviation of mean) among the three evaluated groups ($P<0.05$). However, high variation was observed in the mite numbers in all three groups, as this ascertained by the coefficient of variation (CV). This variation appears to be higher in group C ($CV_A$= 42,58%, $CV_B$= 53,52% and $CV_C$=66,09%).

Fall rates of mites in group A ranged from 14,00% to 31,93% (mean 21,53%), in group B from 10,86 to 64,97% (mean 34,50%) and in group C from 22,83% to 53,40 (mean 37,00%). Evaluation of data shows that fall rates of the mites (in terms of mean %) is significantly higher in group C ($P<0,05$), as well as in group B ($P<0,07$), when these were compared to group A.

It is concluded that the mite burden in the colonies of all three evaluated groups was relatively low. The variation that was recorded among colonies of the same group could be attributed to the other factors and also to the existing differences in host defense mechanisms.